

The effect of postmortem aging and location on tenderness of steaks from beef
Semitendinosus and Longissimus lumborum

by

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Abstract

The objective of this study was to determine the effect of extended postmortem aging (**DOA**), steak location (**LOC**), and dietary treatment (**TRT**) on cooked meat tenderness, sarcomere length, and myofibrillar protein degradation of steaks from the *Semitendinosus* (**ST**) and *Longissimus lumborum* (**LL**). Crossbred feedlot steers ($n = 40$; initial body weight 638 ± 29 kg) were fed 45 d with the following diets: a control diet, control diet with microalgae meal, microalgae meal and antioxidants fed at the beginning of feeding, and microalgae meal with antioxidants fed during the final 10 d of feeding. The ST and LL were removed from carcasses. The ST was fabricated into 10 steaks, which were paired with an adjacent steak and assigned 5 LOC; LOC 1 was the most proximal and LOC 5 was the most distal. Each LOC was randomly assigned an aging period of 7, 14, 28, 56 or 112 d. The 6 most posterior steaks of the LL were paired with an adjacent steak and assigned 3 locations; LOC 1 being the most anterior and LOC 3 the most posterior. Each LOC of the LL was randomly assigned an aging period of 7, 28, or 112 d. Shear force, sarcomere length, muscle fiber type and size, postmortem proteolysis, and calpain activity were measured across aging periods for each LOC. Improved Warner-Bratzler shear force (**WBSF**) values were detected throughout the 112 d aging period for both ST and LL steaks (quadratic; $P < 0.01$). The largest decrease in shear force occurred between d 7 and 28 for LL and ST steaks. Shear force decreased ($P < 0.01$) from LOC 1 to LOC 5 (proximal to distal) in ST steaks. Steak LOC 5 had the longest sarcomeres over LOC 1, 2, and 3 on d 7, 14, and 28 ($P < 0.01$) in the ST; LOC 4 and 5 also had a greater percentage of Type I fibers ($P < 0.01$). Muscle fiber size in ST steaks decreased ($P = 0.01$) from LOC 1 to LOC 5. As DOA increased, intact calpain-1 decreased (quadratic; $P < 0.01$), with intact calpain-1 completely disappearing by d 56 and d 28 in the ST and LL, respectively. Intact desmin and troponin-T decreased throughout the

112 d in ST and LL steaks (linear; $P \leq 0.03$). Degraded desmin-38 kDa increased ($P < 0.01$) between d 14 and d 28; however, degraded desmin-38 kDa did not continue to degrade ($P = 0.76$) from d 56 to d 112 in ST steaks. Degraded desmin-35 kDa content, however, continued to increase through d 112 ($P < 0.01$). Muscle fiber size and type along with sarcomere length played a substantial role in tenderness differences in steak LOC, whereas calpain and proteolytic activity played a substantial role across DOA.

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Dedication

To my parents: Bill and Susan Matney. Thank you for your unwavering support, instilling in me a never ending faith in the Lord, encouraging me to follow my dreams, and loving me every step of the way.

Chapter 1 - Introduction

The 2010 National Beef Quality Audit (**NBQA**) reported eating satisfaction (tenderness and flavor), was the second most important factor for retailers (Igo et al., 2011). Tenderness is such a prominent factor that Miller et al. (2001) reported consumers would pay \$1.23 per kg more for a “guaranteed tender” product. It has been well established extended postmortem aging improves meat tenderness. The 2010 NBQA reported an aging range of 1 to 358 d postmortem (Igo et al., 2011); however, past literature has only reported aging to d 70 postmortem (Phelps et al., 2016a) in ST steaks and 63 d in LL steaks (Karney et al., 2014). Previous literature has commonly attributed meat tenderness variability to proteolysis, connective tissue, and sarcomere length (Koohmaraie, 2004).

The cause of improved tenderness throughout postmortem aging has been attributed to myofibrillar degradation (Koohmaraie and Geesink, 2006). Phelps et al. (2016a) showed WBSF values decreased through d 70 in the ST, whereas Karney et al. (2014) showed WBSF values decreased through d 70 in the ST. The mechanisms behind the tenderness increase throughout postmortem aging has not been fully explored, as Phelps et al. (2016a) showed calpain activity through d 70 in ST steaks.

Koohmaraie and Geesink (2006) also suggested a sampling location effect on muscle tenderization, thereby providing a more tender product in various locations throughout the muscle. These factors have commonly been measured in relation to postmortem aging and location of the *Longissimus lumborum* (**LL**) due to its high market value; however, fewer studies have examined the tenderness properties of the *Semitendinosus* (**ST**; Shackelford et al., 1997; Rhee et al., 2004; Phelps et al., 2016a). Phelps et al. (2016a) studied the location gradient in the ST and showed increased tenderness from the proximal to distal portion of the muscle, which

could be attributed to a decreased muscle fiber size and increased type I fibers in the proximal to distal locations.

Omega-3 fatty acids are a family of polyunsaturated fatty acids which have been shown to prevent heart disease and cancer (Simopoulos, 1999). The demand for increased omega-3 fatty acids in red meat by consumers have pushed dietary supplementation of flaxseed and fish oil. Recently, algae has been included in animal diets to increase omega-3 fatty acids (Nute et al., 2007; Phelps et al., 2016b); however, the effect of tenderness across aging time and location have not been reported when including algae into the animal diet.

Postmortem aging, intramuscular tenderness gradient, and dietary treatments have all been measured independently in various studies; however, the interaction of these effects when steaks are aged for 112 d have yet to be determined. Therefore, the objective of this study was to determine the effect of extended postmortem aging up to d 112, steak location, and dietary supplementation of algae on cooked meat tenderness and myofibrillar protein degradation in ST and LL steaks.

Chapter 2 - Literature Review

Importance of Tenderness

The three major factors which influence meat palatability are tenderness, juiciness, and flavor (Koochmaraie and Geesink, 2006). Of these three palatability traits, tenderness is the most important trait influencing consumer palatability (Savell et al., 1987; Smith et al., 1987; Miller et al., 2001). According to the 2010 National Beef Quality Audit (**NBQA**), the second most important consideration for retail markets and food service providers when choosing meat to provide the consumer was eating satisfaction, falling only behind food safety. Eating satisfaction was defined to include tenderness and flavor (Igo et al, 2011). However, “inadequate tenderness” was the most important consideration for retailers in the 2000 NBQA (Roeber et al., 2002) and the third most important concern for retailers in the 2005 NBQA (Smith et al., 2005). Miller et al. (2001) established the threshold as to which consumers valued tenderness, according to Warner-Bratzler Shear Force (**WBSF**) values, as well as, the amount consumers were willing to pay for a “guaranteed tender” product. The “guaranteed tender” product used in the study was a fictitious level of tenderness at which in which consumers were willing to pay more for a product. It was reported that consumers were willing to pay \$1.23 per kg more for guaranteed tender product compared to the toughest classification of steak.

Factors Influencing Tenderness

Postmortem Aging

In the realm of meat science, it is widely reported that postmortem aging increases tenderness. The relationship between postmortem aging and tenderness has been reported for over a century, Lehmann (1907) indicated an improvement in meat tenderness during the storage of meat at refrigerated temperatures. Moran and Smith (1929) defined aging, beginning 24 h

postmortem, as the practice of storing meat beyond the normal time to enhance tenderness. The 2010 National Beef Tenderness Survey reported an average 20.5 d of aging period for all retail beef cuts and a range of 1 to 358 d of postmortem aging. Whereas the food service sector indicated a longer average aging period of 28.1 d and a much smaller range of 9 to 67 d of aging. The strip loin, which is comprised of the LL was aged an average of 21.6 d postmortem. In the survey, 95.95% and 97.29% of beef LL steaks were considered very tender (WBSF < 31.4 N) or tender (31.4 N < WBSF < 38.3 N) in the retail and food service sectors, respectively. In the retail sector 36.2% of the top loin steaks were aged less than 14 d, while 15.8% were aged less than 14 d in the food service sector (Guelker, 2013). Postmortem aging times have varied in studies, as studies have utilized 28 d (Gruber et al., 2006), 56 d (Juarez et al., 2009), 63 d (Karney et al., 2014) and 70 d (Phelps et al., 2016a).

The changes in meat tenderness are produced by proteolytic action (Hoagland et al., 1917; Penny; 1980; Koohmaraie, 1994). Calpain enzymes are the main proteolytic system which is responsible for meat tenderization (Koohmaraie, 1994; Pomponio, 2008). The calpains are calcium dependent proteases which require calcium from the sarcoplasmic reticulum, which is released during rigor mortis (Calkins and Seideman, 1988). The optimal activity for calpains occurs at a neutral pH. Three types of calpains are present in skeletal muscle, calpain-1, calpain-2, and p94. Additionally, calpastatins are present in the skeletal muscle and are inhibitors to the calpains (Koohmaraie & Geesink, 2006). The calcium dependent proteases: calpain-1 and calpain-2 each require different levels of calcium to activate the proteases, causing the calpain systems to be activated at different calcium levels postmortem. Calpain-1 is activated by 1 μ m of calcium and Calpain-2 is activated by 1 mm of calcium.

Location

For the past 60 years, tenderness within a muscle has been extensively researched and studies have shown a wide range of intramuscular tenderness variation (Ginger and Weir, 1958). The variation in tenderness throughout a muscle is important to recognize, as the sampling position has a significant effect on tenderness (Alsmeyer et al., 1965). Additionally, this knowledge has become increasingly more industry applicable, as the beef industry moves toward innovative cutting strategies. Research regarding intramuscular tenderness variation will continue as the industry strives to provide consumers with a consistent product (Shackelford et al., 1997). Many of these innovative cuts will originate from lower valued portions of the carcass, such as the round and chuck. Senaratne et al. (2010) examined the M. adductor femoris, M. biceps femoris, M. gracilis, M. pectineus, M. sartorius, M. semimembranosus, ST, M. vastus intermedius, M. vastus medialis, and M. vastus lateralis to determine the tenderness gradient within the muscles. It was found that there was a significant tenderness gradient in the M. adductor femoris, M. biceps femoris, M. semimembranosus, M. semitendinosus, and M. vastus lateralis. In order to obtain the most consistent eating quality, cuts with large intramuscular tenderness gradients should be marketed as steaks.

Bratcher et al. (2005) examined the location effect on muscles in the round and chuck, specifically the infraspinatus, triceps brachii-lateral head, triceps brachii-long head, serratus ventralis, complexus, splenius, rhomboideus, vastus lateralis, and rectus femoris. The study concluded the tenderness gradient and location should be considered when merchandising the complexus, rectus femoris, and vastus lateralis, while other muscles would be more suitable to market as whole muscle innovative cuts.

As Senaratne et al. (2005) and Bratcher et al. (2005) stated there are multiple muscles throughout the carcass with intramuscular tenderness gradients. More specifically, intramuscular tenderness variation has been reported throughout the ST muscle. Shackelford et al. (1997) reported a decrease in tenderness acceptability throughout the ST muscle, as there was a large variation in the number of samples rated “slightly tender” in the most proximal (48%) and distal (96%) portions of the ST. Research has also shown a tenderness gradient between the extremity and mid portions of the muscle (Reuter et al., 2002; Denoyelle and Lebihan, 2003; Janz et al., 2006)

A considerable amount of research has been conducted examining the tenderness gradient in the LL. A tenderness gradient in the LL was previously reported by Gariépy et al. (1990) and Martin et al. (1971). Most recently, Derington et al. (2011) examined slice shear force and WBSF in relation to the tenderness gradient. The study concluded the most anterior (29.0 N) portion of the muscle had decreased WBSF values compared to the most posterior (34.1 N) portion of the muscle. Tenderness gradients could result from varying chilling rates throughout the muscle (Janz et al., 2006), carcass suspension (Herring et al., 1965), fiber type (Gariépy et al., 1990; Phelps et al., 2016a), and connective tissue (Torrescano et al., 2003).

Assays

Warner Bratzler Shear Force

Warner-Bratzler shear force is an instrumental tenderness evaluation which was developed in the 1920's to obtain an objective, repeatable measure of tenderness (Warner, 1928). Numerous studies have been conducted to compare this objective tenderness measurement to trained panel sensory tenderness scores. Shackelford et al. (1995) examined the relationship between the shear force value and the trained panelist tenderness scores for ten muscles, psoas

major, infraspinatus, triceps brachii, LL, ST, gluteus medius, supraspinatus, biceps femoris, semimembranosus, and quadriceps femoris. Differences in the shear force values for the psoas major and infraspinatus were detected compared to the other muscles, however, shear force was unable to detect differences in the other muscles examined. Additionally, trained panelists indicated the highest tenderness scores for the psoas major and infraspinatus. However, panelists were also able to detect differences in the other muscles examined. Panelists scored triceps brachii and LL as the second most tender muscles. The ST, gluteus medius, and supraspinatus were the third most tender muscle group. Finally, the fourth most tender group of muscles consisted of the biceps femoris, semimembranosus, and quadriceps femoris. Therefore, the relatability of shear force values to trained sensory panelists could not be determined. Miller et al. (1995) compared shear force values to panelist scores and determined the threshold consumers were able to determine a difference in tenderness of a sample was 1.0 kg. (Miller et al., 1995).

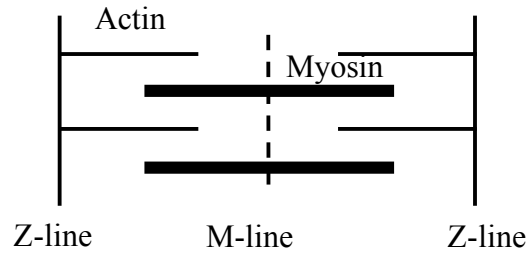
Sarcomere Length

According to Merriam-Webster dictionary (2016), sarcomere length is defined as the repeating structural unit of striated muscle fibers. Sarcomeres are the smallest unit of muscle and are comprised mainly of the proteins actin and myosin (Fig. 1). Actin is the main protein in the thin filament, while myosin is the main protein of the thick filament of the sarcomere. Sarcomeres comprise myofibrils, which are repeating sarcomere units. Myofibrils are surrounded by the sarcolemma and run the entire length of a muscle fiber. The bands of each myofibril are aligned throughout the myofibril which contributes to the striated muscle appearance. Myofibrils then comprise a muscle fiber. Muscle fibers can have various cross-sectional areas and are composed of differing amounts of myofibrils.

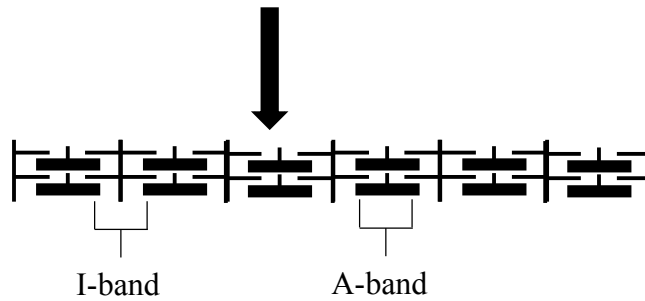
Muscle bundles are a group of muscle fibers surrounded by epimysium. This epimysium and the amount of muscle fibers in a muscle bundle determines the texture of a muscle. Muscle bundles are combined to form a muscle.

Figure 1. Diagram of the organizational structure of muscle, showing the relationship between **a) sarcomere**, **b) myofibrils**, **c) muscle fibers**, **d) muscle bundles**, and **e) muscle** (Aberle et al., 2001).

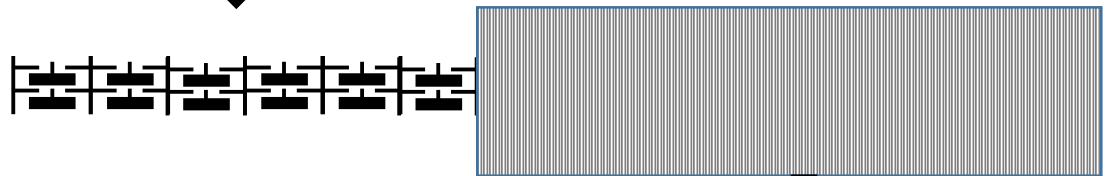
a) sarcomere



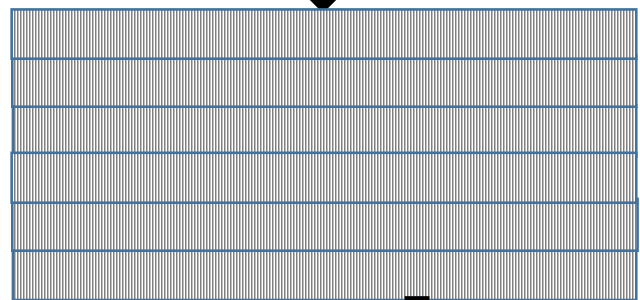
b) myofibril



c) muscle fiber



d) muscle bundle



e) muscle



During rigor mortis sarcomere length shortens and meat toughens during the first 24 h postmortem (Wheeler and Koohmaraie, 1994; Koohmaraie et al., 1996). Sarcomere length reflects the amount of actomyosin formation or disassociation at times after slaughter and is measured from z-line to z-line (Asghar and Yeates, 1978). Actomyosin is the cross-bridge of actin and myosin interaction, the filaments pull the Z disks closer to the myosin filament, causing decreased sarcomere length. Muscles which are stretched or shortened less during rigor mortis are more tender, compared to those with shorter sarcomere lengths (Koohmaraie et al., 1996). As the actin and myosin are spread throughout the entire sarcomere length, the protein in the sarcomere is less dense, producing a more tender product (Aberle et al., 2001).

Sarcomere length measurement has evolved over time, as the methodology has changed in the past 30 years. Previous literature has measured sarcomere length in cooked and uncooked samples at various postmortem aging times. Most recently, Grayson et al. (2016) measured sarcomere length using the protocol by Cross et al. (1981) and Wheeler et al. (2002), which used the laser diffraction method, utilizing the cores used for WBSF. Cross et al. (1981) compared three sarcomere techniques, the laser diffraction method, filar micrometer method, and shearicon/size analyzer method. The authors reported no significant difference in sarcomere length measurement between the three methods.

Smulders (1990) evaluated sixty beef carcasses with varying pH decline rates for sarcomere length, sensory tenderness, and WBSF. Carcasses were cut into subprimals and the short loin was used for tenderness evaluations. One steak was aged for 14 d, five steaks were then frozen and used for WBSF, sensory evaluation, and sarcomere length. Sarcomere length was measured using the laser diffraction method, in which, muscles were fixed with glutaraldehyde and muscle bundles were teased from the steak; fifteen samples per steak were

measured. The authors reported a 0.54 correlation coefficient between sarcomere length and sensory panel tenderness and a -0.46 correlation between sarcomere length and aged steak WBSF measurements, with sarcomere length ranging from 1.53-2.08 μm . Previous literature has reported the correlation (-0.50) between cooked sarcomere length and WBSF values (Bouton et al., 1973). Lewis et al. (1977) reported a relatively low correlation between cooked sarcomere length and WBSF values (-0.33). These correlations suggest that sarcomere length plays a role in meat tenderness.

Fiber Type and Cross Sectional Area

Skeletal muscle is composed of a combination of fibers, and these muscle fibers are either red or white fibers. Muscle fibers are classified by the amount of glycogen a fiber contains and the contraction speed of the fiber. Muscle fiber types are determined by the myosin heavy chain isoforms (Chikuni et al., 2004). Type I, Type IIA, Type IIX, and Type IIB are the four myosin heavy chain isoforms that have been identified in skeletal muscle (Aberle et al., 2001). Chikuni et al. (2004), however, investigated muscle fiber types in bovine muscles and discovered the only myosin heavy chain isoforms present were Type I, Type IIA, and Type IIX. These muscle fibers have different characteristics, as they are classified by how fast the muscle fibers contract, as well as the amount of glycogen in each of the fiber types. Type I fibers are slow twitch, oxidative fibers with a high myoglobin content. The high myoglobin content increases the red appearance of the muscle fiber (Schiaffino et al., 1989). Type IIA fibers are classified as fast-twitch oxidative glycolytic muscle fibers with a high myoglobin content. Finally, Type IIX fibers are muscle fibers which are fast twitch glycolytic fibers with a low myoglobin content (Aberle et al., 2001; Schiaffino et al., 1989). Muscle fiber type present in a muscle is not only

dependent on the species but also the muscle that is being examined. Muscles from different regions of the carcass have different muscle fiber type distributions (Dransfield et al., 2003).

Muscle growth after birth occurs through hypertrophy, which is defined as a thickening of muscle fibers without multiplication of parts (Merriam-Webster, 2016). An increase in cross sectional area is correlated to a decrease in tenderness (Crouse et al., 1991; Chriki et al., 2012). Type IIX fibers are inherently the largest fibers of the three fiber types present in beef due to their glycolytic nature. Whereas Type I fibers are the smallest fibers due to their oxidative properties (Chriki et al., 2012). Dransfield et al. (2003) and Seideman et al. (1987) reported a stronger correlation between fiber type and beef tenderness than fiber area and beef tenderness. The stronger correlation between fiber type and beef tenderness than fiber area and beef tenderness, could be attributed to the nature of each fiber type.

Fiber type and fiber area have been measured since the 1970's, however, staining techniques have become more advanced over time. Schiaffino et al. (1989) discovered a fourth mammalian muscle fiber type through the use of antibodies. Type IIX fibers have similar dehydrogenase activity as type IIA fibers. Schiaffino et al. (1989) indicated type I fibers were fibers staining positive for a BA-D5 antibody. Type IIA fibers stained positive for BF-35 and negative for BA-D5 antibodies. The type IIX fibers would be classified with the type IIA muscle fibers, as they produced a moderately strong dehydrogenase activity, indicating a more oxidative enzyme. Fibers which stained negative for the BF-35 antibody were classified as type IIX. Before these staining techniques were adopted by Schiaffino type IIX fibers were stained the same as type IIA fibers.

Calpastatins

Calpastatins inhibit the amount of calpain activity occurring in a system. The calpastatin system is best demonstrated in callipyge sheep. Callipyge sheep have increased levels of calpastatin activity. Koohmaraie et al. (1995) and Geesink et al. (2001) reported an increase in calpastatin activity, which led to decreased tenderization in the longissimus of the callipyge sheep compared to the longissimus of normal sheep. Geesink and Koohmaraie (1999) examined the effect of calpastatin on postmortem aging. Callipyge lambs have an increased level of calpain and calpastatin activity in muscle. Therefore, the authors utilized normal and callipyge lamb muscle to determine if increased levels of calpastatins in a system decreased the rate of tenderization or if the increase in calpastatins limited the extent to which postmortem proteolysis could occur.

Geesink and Koohmaraie (1999) measured calpain and calpastatin activity at 0, 14, and 56 d postmortem on the biceps femoris muscle using casein to determine calpain activity, the standard calpain assay was used to express the activity in standard units. The authors reported a significant day of aging and phenotype effect on calpain-1 activity. The calpain-1 levels decreased throughout postmortem days of aging. The callipyge sheep produced a lower rate of autolysis compared to normal muscle. This suggests that the increased levels of calpastatin inhibited postmortem proteolysis in the muscle. The authors also reported a stable calpain-1 level throughout the postmortem aging time. The stability of calpain-1 levels is due to insufficient calcium ions needed to activate the enzyme (Dransfield, 1993). An inverse relationship exists with calpastatin activity and tenderness, as calpastatin increases, tenderization decreases (Koohmaraie et al., 1995).

Calpain Activity

Enzymatic activity plays an important role in meat tenderness (Hoagland et al., 1917). Two calpains, calpain-1 and calpain-2, are involved in postmortem tenderization as they undergo autolysis when calcium is present. Both calpain-1 and calpain-2 are calcium dependent substrates comprised of two subunits with molecular weights of 28 and 80 kDa (Dayton et al., 1976; Dayton et al., 1981; Koohmaraie et al., 2006). The calpain system degrades Z-disks, which account for 90% of tenderization which occurs during postmortem aging (Taylor et al., 1995). The calpain system initiates myofibril degradation by degrading several proteins, desmin, vinculin, *meta*-vinculin, dystrophin, nebulin, titin, troponin-T, and troponin-I. Both intact and autolyzed calpains continue to degrade proteins throughout postmortem aging (Phelps et al., 2016).

Postmortem Proteolysis

Desmin and troponin-T are degraded by the calpain system, which have all been reported to affect meat tenderness (Nowak, 2011). Meat tenderization is described as breaks at the junction of I band and the Z-lines (Koohmaraie et al., 2006). Desmin and troponin-T are two cytoskeletal proteins which indicate the amount of tenderization occurring in the system. The Z to Z line attachments in muscle structure is composed of desmin (Koohmaraie et al., 2006). Additionally, troponin-T, while not located in the Z-disk, (Taylor et al., 1995) has a strong correlation to meat tenderness. Troponin-T is a non-structural protein attached to the actin or thin filament of a sarcomere. The degradation of troponin-T indicates tenderization is occurring as it breaks down other proteins are also breaking down in the process.

Desmin is an intermediate filament that surrounds the Z-lines of the myofibrils (Huff-Lonergan et al., 2010) and it is degraded throughout postmortem aging (Taylor et al., 1995;

Huff-Lonergan et al., 1996; Melody et al., 2004). Degradation of desmin during postmortem aging significantly contributes to tenderness (Koochmaraie and Geesink, 2006). Intact desmin has a molecular weight of 55-kDa, degraded desmin-38 kDa has a molecular weight of 38-kDa, and degraded desmin-35 kDa has a molecular weight of 35 kDa. As desmin begins to degrade, a greater amount of desmin will be present at the 38-kDa polypeptide, which indicates an increased amount of degradation, meanwhile, as meat goes through extended postmortem aging a secondary band appears, at the degraded desmin-35 kDa level (Huff-Lonergan et al., 2010).

The thin filament of skeletal muscle is comprised of troponin-T, this cytoskeletal protein is degraded by calpains and regulates the thin filament during skeletal muscle contraction (Greaser and Gergely, 1971; Huff-Lonergan et al., 2010). Troponin-T is a non-structural protein which has been shown to be degraded by calpain-1 (Huff-Lonergan et al., 2010) and is often associated with the tenderness of beef (MacBride and Parrish, 1977; Huff-Lonergan et al., 2010). Intact troponin-T has a molecular weight of 40 kDa, which is present when muscle is intact. However, throughout postmortem tenderization, polypeptides appear at approximately 28-30 kDa. The appearance of these peptides and the disappearance of intact troponin-T are strongly related to the tenderization of skeletal muscle, even though research has not reported troponin-T to have a direct effect on meat tenderness (Huff-Lonergan et al., 1996; Huff-Lonergan and Lonergan, 1999; Huff-Lonergan et al., 2010).

As stated above, Geesink and Koochmaraie (1999) examined the effect of postmortem proteolysis on postmortem aging. The effect of increase calpastatins on the amount of postmortem proteolysis was examined using callipyge and normal lambs. Desmin, troponin-T, titin, nebulin, dystrophin, vinculin, alpha-actin, and the myosin heavy chain were measured at 1, 3, 7, 21, 42, and 56 d postmortem using SDS-PAGE and immunoblotting. Authors reported

desmin degraded at a slower rate in the callipyge lamb than normal muscle. The percentage of desmin remaining after d 21 was 19.5% in normal muscle compared to 53.6% in callipyge muscles. Similar results were obtained for troponin-T degradation, as troponin-T degraded at a slower rate in callipyge muscle compared to the normal muscle. A greater amount of fragments were visible at 30 kDa (degraded troponin-T band density) for troponin-T in normal muscle after 56 d, compared to the callipyge muscle at 56 d postmortem. The authors contributed differences in desmin and troponin-T degradation to differences in calpastatin activity in normal and callipyge muscles.

Postmortem Aging

Warner Bratzler Shear Force

It has been well established by previous literature, postmortem aging increases tenderness. Warner-Bratzler shear force values decrease as aging time increases throughout the whole carcass (Rhee et al., 2004; Gruber et al., 2006). However, studies have concluded postmortem aging has different effects on various muscles throughout the carcass. Gruber et al. (2006) reported a decrease in WBSF value as aging time increased in the biceps femoris, complexus, gluteus medius, infraspinatus, longissimus dorsi, psoas major, rectus femoris, semimembranosus, ST, serratus ventralis, spinalis dorsi, supraspinatus, tensor fasciae latae, teres major, triceps brachii, vastus lateralis, and vastus medialis. While all muscles responded to postmortem aging, it is important to note muscles respond to aging at different rates. More specifically to this study, the USDA Select ST had a 1.6 kg aging response with 59.5% and 91.9% of aging completed by d 10 and d 21, respectively. The USDA Select LL had a 2.5 kg response to aging with 49.1% and 86.6% of aging completed by d 10 and d 21, respectively.

In a 2009 study by Juárez et al., the ST and LL were aged for 0, 7, 14, 21, 28, 35, 42, 49, and 56 d in order to demonstrate the various aging times of beef throughout the industry. The ST and LL showed decreased shear force values from d 7 to 56 (33.6% and 10.1%), respectively. However, there was no decrease in WBSF values detected past d 28 in the ST and the LL, consistent WBSF values were reported at d 56 and d 35. Phelps et al. (2016a) showed an 18.3% improvement in shear force from d 7 to d 70, in the ST with a final shear force value of 4.4 kg on d 70, most of the decrease in shear force values occurred by d 21, there was improvements in tenderness through d 70.

Sarcomere Length

Sarcomere length is the measurement of contractile proteins which is determined during rigor mortis, the time period directly after slaughter (Asghar and Yeates, 1978). Starkey et al. (2015) utilized sarcomere length to evaluate the variation in shear force values in the lamb longissimus muscle at 0, 7 and 14 d postmortem aging. The authors measured sarcomere length using the microscope methodology, in which sample was homogenized with a sucrose solution and placed onto a microscope and 10 sarcomere sections were evaluated for 10 myofibrils. The authors reported no difference in sarcomere length across aging periods, and reported a low sarcomere variation accounted for by aging ($R^2 = 1.50\%$). These results are in accordance to previous literature and meat science knowledge, as sarcomere length has been reported to not be variable across aging times.

Geesink et al. (2001) also researched the effect of aging on sarcomere length in the lamb longissimus. Ten lambs were utilized to measure the effect of sarcomere length and free calcium postmortem on tenderness in the lamb longissimus. Authors used electron microscopy, fixed the sample in a 2.5% glutaldehyde in 0.1 M cacodylate buffer then stained with uranyl acetate and

lead citrate. Sarcomere length was measured on 50 sarcomeres per sample and shrinkage was measured in comparison to the A band. The authors did not show a difference in sarcomere length between 1 and 7 d postmortem, as sarcomere lengths were $1.87 \pm 0.06 \mu\text{m}$ and $1.77 \pm 0.08 \mu\text{m}$, respectively.

In contrast, King et al. (2003) examined the effect of chilling and cooking rates on the tenderness of beef longissimus thoracis and triceps brachii, long head for 1 or 14 d postmortem. Sarcomere length was measured in both raw and cooked steaks using the laser method, in which a laser was passed through the sarcomere and the diffraction pattern was measured and calculated using the equation described by Cross et al. (1981). Authors reported no difference in sarcomere length across aging period for the longissimus thoracis. However, there was an increase in sarcomere length of the triceps brachii between 1 and 14 d postmortem 1.70 ± 0.02 and 1.74 ± 0.02 , respectively. This is a relatively small, yet statistically different difference in sarcomere length. Previous literature has suggested this difference could be in sampling error as only 36 sarcomeres per cooked sample were measured for sarcomere length. Geesink (2001) suggested the difference in sarcomere length across aging periods could be attributed to sampling error within each study.

Fiber Type and Cross Sectional Area

Fiber cross sectional area or fiber diameter plays an important role in beef tenderness in the early stages of post-mortem storage, however, the importance of fiber diameter diminishes throughout aging time (Crouse et al., 1991). Muscle fiber type is attributed to several background factors such as: age, gender, feeding regimen, breed, and muscle type (Chraki et al., 2013). Similarly, cross sectional area is also attributed to various antemortem factors such as age, feeding, breeding, exercise, and gender (Picard et al., 2007; Chraki et al., 2013). Muscle

fiber type and size are determined antemortem, therefore, muscle fiber type and size do not change during postmortem aging.

Calpain Activity

Previous literature has reported a correlation between shear force values and calpastatin and calpain activity. Calpastatin, as discussed earlier is an endogenous inhibitor for the calpain system. However, Whipple et al. (1990) examined the longissimus muscle in crossbred cattle at 14 d postmortem, the authors did not report a significant correlation between calpain-1 and calpain-2 and WBSF values.

Phelps et al. (2016a) used casein zymography to examine the effect of calpain activity on beef tenderness through 70 d postmortem on ST steaks. Calpains were measured at 7, 14, 21, 42, and 70 d. The authors reported intact calpain-1 decreased throughout the aging period until d 42 with the greatest reduction occurring between d 7 and d 14, with smaller reductions occurring between d 14 and d 42. Autolyzed calpain-1 showed no change between d 7 and d 42. Furthermore, the amount of autolyzed calpain-2 significantly decreased at d 70. This information is cause for further exploration of the role calpain-2 plays in the tenderization of muscle through postmortem aging.

Postmortem Proteolysis

Desmin and troponin-T degradation were measured up to d 70 postmortem by Western-Blot analysis. The relative band density of intact desmin and troponin-T were evaluated up to d 70 in ST steaks, with measurements taken at d 7, 14, 21, 42, and 70 d postmortem. The relative band density of intact desmin and troponin-T decreased linearly through d 70, while degraded desmin increased linearly from d 7 to d 70 (Phelps et al., 2016a).

In contrast, Starkey et al. (2015) measured desmin activity (degraded/intact ratio) in the lamb longissimus for three different aging periods (1, 7, and 14 d). The authors reported a significant increase in degraded desmin from d 1 to d 7, however, after d 7 of aging there was no difference in desmin degradation. The difference in the postmortem aging effect on degraded desmin could be attributed to species or muscle differences.

Location

Warner Bratzler Shear Force

Various studies have examined the intramuscular tenderness gradient of various muscles (Rhee et al., 2004; Derrington et al., 2010; Phelps et al., 2016a). Studies have reported differing intramuscular gradients throughout the ST. Phelps et al. (2016a) examined tenderness within the ST at 10 different steak locations. The middle portion of the muscle produced a more tender product compared to the proximal and distal extremities of the muscle. Reuter et al. (2002) found similar results, as the middle 4 steaks shear force decreased by 13% and 11% compared to the 4 most proximal and 3 most distal steaks, respectively. In total contrast, previous literature reported a decrease in the shear force values from the proximal to distal ends. Rhee et al. (2004) and Shackelford et al. (1997) reported decreased shear force values from proximal to distal portions of the ST.

An intramuscular tenderness gradient has also been determined in the LL. Rhee et al. (2004) reported an increase in tenderness in the middle location compared to the anterior and posterior locations. However, similar to the Derrington et al. (2010) reported the most tender portion of the muscle was the anterior end.

Sarcomere Length

The location of a steak within a muscle has an effect on the sarcomere length in certain muscles (Rhee et al., 2004). Authors measured sarcomere length using the laser diffraction method for the longissimus, psoas major, gluteus medius, semimembranosus, adductor, biceps femoris, ST, rectus femoris, triceps brachii, infraspinatus, and supraspinatus. More specific to this study, the sarcomere length of the ST and longissimus was measured. The entire longissimus was used in this study, and the posterior location corresponded to the LL used in this study. Muscles were aged for 14 d postmortem and frozen, then cut into steaks and assigned a location allocated for sensory or WBSF measurement. Steaks utilized for WBSF were also used to measure sarcomere length. Muscles were divided into three locations per muscle and sarcomere length was measured for each location of the muscle.

Sarcomere length was affected by location in certain muscles. Authors showed a difference in sarcomere length of the locations in the ST and LL. As the ST progressed from the proximal to distal end the sarcomere length increased by 27%. However, the longissimus produced a 2% decrease in sarcomere length in the most anterior portion of the muscle compared to the most posterior and middle portion of the muscle. However, this variation was across the entire longissimus muscle. No difference in sarcomere length of the most posterior and middle steaks in the LL was detected. The variation of sarcomere length was greater in the ST than the longissimus, which could indicate a larger tenderness variation in the ST.

Fiber Type and Cross Sectional Area

Fiber type and cross-sectional area has been measured in numerous studies, however, few studies have examined the intramuscular gradient in relation to cross sectional area in the LL or ST. Ishii et al. (1995) measured the cross-sectional area and fiber type in different locations of

the LL, M. deltoideus, M. rectus thoracis, M. psoas minor, M. tensor fasciae latae, ST, and M. gastrocnemius of four steers to two different finishing ages, 18 or 27 months of age with finishing weights groups of 650 and 750 kg, respectively. The ST was measured in 4 sampling positions, with site 1 being the most proximal section, site 2 as the most distal portion, and sites 3 and 4 being located in the middle section, with 3 as the superficial position and 4 as the profound portion of the muscle. The authors reported a decrease in cross sectional area in the middle portion of the muscle compared to the distal and proximal portions of the muscle in the ST. The LL was measured at the 8th thoracic vertebrae for the anterior portion, as well as the 3rd lumbar vertebrae for the posterior portion of the muscle. Muscle fiber cross-sectional area decreased in the posterior portion of the muscle in 3 of the 4 animals examined for cross-sectional area.

Phelps et al. (2016a) examined the proximal and distal portions of the muscle for muscle fiber type and cross-sectional area in the M. semitendinosus. An increase in muscle fiber cross sectional area in the ST was reported when comparing the proximal to distal locations. The average cross sectional area decreased in the distal portion of the muscle for all three fiber types, 33% smaller Type I, 24% smaller Type IIA, and 35% smaller Type IIX muscle fibers. Furthermore, the fiber type distribution was measured for the ST and there was an increase in Type I and Type IIA fiber percentage, however, there was a decreased percentage of Type IIX fibers. Thereby, cross-sectional area is typically positively correlated to tenderness values. This was observed as the most proximal portion of the muscle had an increased shear value compared to the most distal portion.

Calpain Activity

Steak location has not been shown to have an impact on calpain activity. Phelps et al. (2016a) hypothesized an increased calpain activity in locations with a higher percentage of type I or type IIA muscle fibers. The authors hypothesized this utilizing the knowledge that calpain-1 and calpain-2 are generally accepted to have a constant ratio across muscles. Furthermore, according to Ouali and Talmant (1990) type I or type IIA muscle fibers contain more calpain-2 than type IIX muscle fibers. Therefore, it could be implied that locations with an increased percentage of type I or type IIA muscle fibers could contain a greater amount of calpains than the other locations. This did not hold true in the Phelps et al. (2016a) study, as there was no difference in intact or autolyzed calpain-1, intact or autolyzed calpain-2, and total calpains.

Postmortem Proteolysis

Desmin and troponin-T degradation has commonly been measured, however, few studies have measured the effect of the intramuscular gradient on proteolytic activity. Rhee et al (2004) measured the desmin activity in eleven beef muscles, including but not limited to the LL, ST, psoas major, gluteus medius, biceps femoris, semimembranosus, abductor, and the rectus femoris. Each muscle was divided into three locations, location 1 being the most proximal or anterior portion and location 3 as the most distal or posterior portion; the intramuscular variation was measured among the three locations in each muscle. The gluteus medius was divided into two locations, location 1 being the most posterior and location 2 being the most anterior portion. The cores which were used for WBSF were used to examine desmin degradation percentage. The psoas major, gluteus medius, biceps femoris, semimembranosus, abductor, and the rectus femoris all presented desmin degradation percentage differences, as the percentage of desmin degradation increased from the proximal to distal locations. Muscles varied an average of 24.7%

from the most proximal or posterior portion to the most distal or anterior portion of the muscle. The authors showed the locations with increased desmin degradation in the semimembranosus were closely correlated to WBSF value differences.

While some muscles did produce differences in desmin degradation across locations, there was no location difference in desmin degradation percentages in the ST or LL (Rhee et al (2004). Phelps et al. (2016a) was in agreement with Rhee, as there was no difference in desmin and troponin-T degradation in ST steaks across 10 steak locations.

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Chapter 3 - The effect of postmortem aging and location on tenderness of steaks from beef *Semitendinosus* and *Longissimus lumborum*

Abstract

The objective of this study was to determine the effect of extended postmortem aging (DOA), steak location (LOC), and dietary treatment (TRT) on cooked meat tenderness, sarcomere length, and myofibrillar protein degradation of steaks from the *Semitendinosus* (ST) and *Longissimus lumborum* (LL). Crossbred feedlot steers ($n = 40$; initial body weight 638 ± 29 kg) were fed 45 d with the following diets: a control diet, control diet with microalgae meal, microalgae meal and antioxidants fed at the beginning of feeding, and microalgae meal with antioxidants fed during the final 10 d of feeding. The ST and LL were removed from carcasses. The ST was fabricated into 10 steaks, which were paired with an adjacent steak and assigned 5 LOC; LOC 1 was the most proximal and LOC 5 was the most distal. Each LOC was randomly assigned an aging period of 7, 14, 28, 56 or 112 d. The 6 most posterior steaks of the LL were paired with an adjacent steak and assigned 3 locations; LOC 1 being the most anterior and LOC 3 the most posterior. Each LOC of the LL was randomly assigned an aging period of 7, 28, or 112 d. Shear force, sarcomere length, muscle fiber type and size, postmortem proteolysis, and calpain activity were measured across aging periods for each LOC. Improved shear force values were detected throughout the 112 d aging period for both ST and LL steaks (quadratic; $P < 0.01$). Shear force decreased ($P < 0.01$) from LOC 1 to LOC 5 (proximal to distal) in ST steaks. Steak LOC 5 had the longest sarcomeres over LOC 1, 2, and 3 on d 7, 14, and 28 ($P < 0.01$) in the ST; LOC 4 and 5 also had a greater percentage of Type I fibers ($P < 0.01$). Muscle fiber size in ST

steaks decreased ($P = 0.01$) from LOC 1 to LOC 5. As DOA increased, intact calpain-1 decreased (quadratic; $P < 0.01$), with intact calpain-1 completely disappearing by d 56 and d 28 in the ST and LL, respectively. Intact desmin and troponin-T decreased throughout the 112 d in ST and LL steaks (linear; $P \leq 0.03$). Degraded desmin-38 kDa increased ($P < 0.01$) between d 14 and d 28; however, degraded desmin-38 kDa did not continue to degrade ($P = 0.76$) from d 56 to d 112 in ST steaks. Degraded desmin-35 kDa content, however, continued to increase through d 112 ($P < 0.01$). Muscle fiber size and type along with sarcomere length played a substantial role in tenderness differences in steak LOC, while calpain and proteolytic activity played a substantial role across DOA.

Introduction

The 2010 National Beef Quality Audit (NBQA) reported eating satisfaction (tenderness and flavor), was the second most important factor for retailers (Igo et al., 2011). Miller et al. (2001) reported consumers would pay \$1.23 per kg more for a “guaranteed tender” product. It has been well established extended postmortem aging improves meat tenderness. The 2010 NBQA reported an aging range of 1 to 358 d postmortem (Igo et al., 2011); however, past literature has only reported aging to d 70 postmortem (Phelps et al., 2016a) in *Semitendinosus* (ST) steaks and 63 d in *Longissimus lumborum* (LL) steaks (Karney et al., 2014).

Phelps et al. (2016a) showed WBSF values decreased through d 70 in the ST, whereas Karney et al. (2014) showed WBSF values decreased through d 49 in the LL. Mechanisms behind the tenderness increase throughout postmortem aging has not been fully explored, as Phelps et al. (2016a) showed calpain activity through d 70 in ST steaks.

Koohmaraie and Geesink (2006) also suggested a sampling location effect on muscle tenderization, thereby providing a more tender product in various locations throughout the muscle. Factors associated with postmortem aging and location of the LL due to its high market value have been measured; however, fewer studies have examined the tenderness properties of the ST (Shackelford et al., 1997; Rhee et al., 2004; Phelps et al., 2016a). Literature has shown an increase in tenderness from the proximal to distal locations, however, the reason behind the tenderness gradient has not been fully explored (Phelps et al., 2016a).

The demand for increased omega-3 fatty acids in red meat by consumers, to prevent heart disease and cancer (Simopoulos, 1999) have pushed dietary supplementation of flaxseed and fish oil. Recently, algae has been included in animal diets to increase omega-3 fatty acids (Nute et al., 2007; Phelps et al., 2016b).

Postmortem aging, intramuscular tenderness gradient, and dietary treatments have all been measured independently in various studies; however, the interaction of these effects when steaks are aged 112 d have yet to be determined. Therefore, the objective of this study was to determine the effect of extended postmortem aging up to d 112, steak location, and dietary supplementation of algae on cooked meat tenderness and myofibrillar protein degradation in ST and LL steaks.

Materials and Methods

Animals and Semitendinosus and Longissimus lumborum Collection

Forty-crossbred steers were bought at a Kansas auction barn and fed at the Kansas State University Beef Cattle Research Center. Animals were fed four dietary treatments consisting of: control diet (**CON**; Table 1), control diet with 100 g·steer⁻¹·d⁻¹ microalgae meal (**ALG**; Alltech Inc., Nicholasville, KY), 100 g·steer⁻¹·d⁻¹ supplemental microalgae meal plus 103 IU/d vitamin E and SelPlex (Alltech Inc.) fed throughout feeding (**AOX**), and 100 g·steer⁻¹·d⁻¹ plus supplemental microalgae meal plus 103 IU/d vitamin E and SelPlex (Alltech Inc.) fed during the final 10 d of feeding (**LATE**). Dietary treatments were fed 45 d before harvest and steers were harvested at approximately 18 mo of age at a commercial processing facility (Creekstone Farms, Arkansas City, KS). Final BW and hot carcass weight of steers were 723 ± 31 kg and 460 ± 22 kg, respectively. Carcasses were chilled for 6-d and the ST and LL (IMPS #171C and #180, respectively) were removed and transported in refrigerated conditions, 2 ± 1°C to the Kansas State University Meats Laboratory (Manhattan, KS).

Semitendinosus and L. lumborum Processing

Distal and proximal portions of the ST muscle were determined and the portion of muscle that was not large enough to be cut into a steak was removed. Each ST was fabricated into 10,

2.54-cm steaks with steak 1 being most proximal and steak 10 being most distal to the steers' body. Steaks fabricated successively were paired (1 and 2, 3 and 4, etc.); therefore, location (LOC) 1 consisted of steak 1 and 2, LOC 2-steaks 3 and 4, LOC 3-steaks 5 and 6, LOC 4-steaks 7 and 8, and LOC 5-steaks 9 and 10. Paired steaks were randomly assigned to 7, 14, 28, 56, and 112 d of aging. Within each pair, steaks were randomly assigned to Warner Bratzler Shear Force (WBSF) or laboratory analysis.

The most anterior portion of the LL, which was not large enough to yield a steak, was removed. The LL was fabricated into ten 2.54-cm steaks, with steak 1 being the most anterior and steak 10 the most posterior. The first four steaks of the anterior portion of the LL were removed and utilized for a shelf-life study not associated with this study. The remaining 6 steaks were paired and assigned a steak LOC as described above with LOC 1 consisted of steak 1 and 2, LOC 2-steak 3 and 4, and LOC 3-steak 5 and 6. Each LOC was randomly assigned to 7, 28, or 112 d of aging, and similar to the ST, steaks were randomly assigned to WBSF or laboratory analysis within a LOC. Steaks from the ST and LL were vacuum packaged and aged at $2 \pm 1^\circ \text{C}$. After each aging period, WBSF steaks were utilized to obtain objective tenderness measurements and laboratory analysis steaks were cubed into $1.27 \times 1.27 \times 2.54$ -cm portions and frozen at -80°C for laboratory analysis.

Warner-Bratzler Shear Force

Warner-Bratzler shear force (WBSF) procedures were conducted according to the American Meat Science Association (AMSA) Meat Cookery and Sensory Guidelines (AMSA, 2015). When steaks reached their assigned time of aging, they were weighed and a thermocouple wire (30-gauge copper and constantan; Omega Engineering, Stamford, CT) was inserted into the geometric center of each steak. Internal temperature was monitored by a Doric Minitrend 205

monitor (VAS Engineering, San Francisco, CA). Steaks were cooked to an internal temperature of 65°C on a Cuisinart Griddler (Cuisinart, Stamford, CT) set to 232°C, removed from the grill, and peak temperature (averaged 71°C) was recorded. After peak temperature was reached, final weight was recorded to determine cooking loss. After a 24-h chill period at $0 \pm 2^\circ\text{C}$, six ST 1.27-cm cores and six 1.27-cm LL samples were taken parallel to the muscle fiber orientation. Samples were sheared using a Warner-Bratzler shear head attached to an Instron testing machine (Model 5569; Instron, Canton, MA) with a 100 kg compression load cell and crosshead speed of 250 mm/min.

Sarcomere Length

Sarcomere length procedures were adapted from the protocol described by Grayson et al. (2016) and adapted from Wheeler et al. (2002). The 6 cooked sample cores from the Warner-Bratzler shear force measurement were frozen at -80°C then frozen in liquid nitrogen. Once frozen, the 6 cooked sample cores were combined and powdered in a Waring blender (Waring Products, Inc., Torrington, CT). A small amount of powdered sample with 50 μl of 0.2M sucrose solution in 0.1 M NaHPO_4 buffer was placed on a microscope slide. Once the sample and sucrose solution were combined, the microscope slide was placed under a Spectra Physics Laser Exciter (Spectra Physics, Inc., Eugene, OK) laser beam positioned 16 cm from the table base. Six diffraction patterns were marked on a piece of paper for each group of sample. Six groups of sample were marked, for a total of 36 sarcomere lengths per sample. Z-lines were marked to determine the length of the sarcomere, and M-line was marked as the center of the sarcomere. Diffraction patterns were scanned into JPEG images and measured using the NIS-Elements Imaging Software (Basic Research, 3.3; Nikon Instruments, Inc.). Once lines were measured, sarcomere length was calculated utilizing the equation published by Cross et al. (1981).

Immunohistochemical Analysis

Immunohistochemical analysis procedures were followed as described by Phelps et al. (2014). Subsamples measuring 1 cm × 1 cm × 2.54-cm were collected from the geometric center of the ST, while LL subsamples were obtained from the geometric center of the medial, medial/lateral, and lateral areas of steaks assigned to laboratory analyses. Tissues were embedded and frozen in tissue freezing medium (Fisher Scientific, Pittsburgh, PA) by submersion in super-cooled isopentane. Five micrometer cryosections were collected and blocked with blocking solution consisting of 10% horse serum and 0.2% TritonX-100 in phosphate buffered saline (**PBS**). Cryosections were incubated in a primary antibody mixture for 1 h containing the following antibodies: 1:500 rabbit α -dystrophin (Thermo Scientific, Waltham, MA), 1:10 supernatant mouse anti-myosin heavy chain (**MHC**) slow, IgG2b (BA-D5; Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA), and 1:10 mouse anti-MHC all but type IIX, IgG1 (BF-35; Developmental Studies Hybridoma Bank). Sections were rinsed with PBS 3-times and incubated with a mixture of 3 secondary antibodies: goat anti-mouse IgG1488 Alexa-Fluor 488, goat anti-rabbit H & L Alexa Fluor 594, and goat anti-mouse IgG2 Alexa Fluor 633 (Invitrogen, Grand Island, NY). After washing with PBS 3-times, slides were cover-slipped and photomicrographs were taken at a 100× working distance magnification with a Nikon Eclipse TI-U inverted microscope equipped with a DS-QiMC digital camera (Nikon Instruments Inc., Melville, NY) and EXFO X-Cite fluorescence illumination system (Excelitas Technologies Corporation, Waltham, MA).

A minimum of 500 fibers from the ST and 200 fibers per area of the LL were analyzed for individual myosin heavy chain form and muscle fiber cross-sectional area (**CSA**) utilizing NIS-Elements Imaging Software (Basic Research, 3.3; Nikon Instruments, Inc.). The α -

dystrophin boarder was utilized to identify the periphery of muscle fibers, which were used for CSA measurements. Fibers staining positive for the BA-D5 antibody were classified as type I fibers. Fibers staining positive for the BF-35 antibody, but negative for BA-D5 were classified as type IIA fibers. Fibers negative for both the BA-D5 and BF-35 antibodies were classified as type IIX fibers (Fig. 1).

Sarcoplasmic Protein Extraction and Casein Zymography

The remaining portion of the laboratory steak was cut into cubes, frozen in liquid nitrogen, and pulverized in a Waring blender (Waring Products, Inc.). Casein zymography methodology were conducted as described by Phelps et al. (2016) and Shackelford et al. (1994). Finely minced 5-g tissue was homogenized in 3 vol (wt/vol) of extraction buffer which consisted of 100 mM Tris-HCl (pH 8.3), 10 mM EDTA, 0.1% (vol/vol) β -mercaptoethanol (**MCE**), 2 mM phenylmethylsulfonylfluoride, and 1.5 μ L of Protease Inhibitor Cocktail (Product #: P8340; Sigma Aldrich, St. Louis, MO). Homogenate was centrifuged at $18,500 \times g$ for 30 min at $4^{\circ}C$ and filtered through 3 cheesecloth layers. Protein concentration of each sample was quantified using the Pierce BCA Protein Assay Kit (Thermo Scientific, Waltham, MA). Samples and buffers were held at $4 \pm 2^{\circ}C$.

A vol equal to 140 μ g/ μ L of protein was mixed with an equal vol of loading dye solution (20% glycerol, 0.75% [vol/vol] MCE, 0.1% [wt/vol] bromophenol blue, and 150 mM Tris-HCl, pH 6.8]). Samples were loaded onto 12.5% nondenaturing polyacrylamide gel electrophoresis casein gels and separated at a constant voltage of 125 V for 3 h. Gels were washed 3 times for 20 min and allowed to incubate for 20 h in a buffer of 50 mM Tris-HCl, 5 mM $CaCl_2$, and 0.1% (vol/vol) MCE, with a pH of 7.5. Gels were stained with Coomassie Brilliant Blue R-250 and imaged with a ChemiDoc-It 415 Imaging System (UVP, Upland, CA). Calpain activity was

determined by measuring the cleared bands in the stained gel (Fig 2a and 3a) and band intensities were measured and recorded with VisionWorksLS Image Acquisition and Analysis Software (UVP).

Desmin and Troponin-T Western-Blot Analysis

Desmin and Troponin-T analyses were conducted using procedures modified by Melody et al. (2004) and described by Phelps et al. (2016a). Two-tenths of a gram of sample was homogenizing in 5 mL of whole muscle protein extraction buffer. The homogenate was centrifuged at $1,500 \times g$ for 15 min at 20°C and a Pierce BCA Protein Assay Kit (Thermo Scientific) was used to determine protein concentration. Samples were combined with tracking dye and 30 µg and 45 µg of protein were loaded onto 10% polyacrylamide separating gels with 5% polyacrylamide stacking gels for troponin-T and desmin analyses, respectively. Proteins were separated at 40 mA and transferred to nitrocellulose membranes (Amersham Hybond-ECL; GE Healthcare Bio-Sciences, Pittsburgh, PA) using a TE77X Semi-dry Transfer Unit (Hoefer) for 1.5 h at a constant 140mA amperage.

Membranes were blocked for 30 min at room temperature and Desmin blots were incubated for 20 h at 4°C in 1:15,000 rabbit anti-desmin antibody (Sigma Aldrich) diluted in 1% NFDM and TBS-T. Troponin-T blots were incubated for 20 h at 4°C in mouse anti-troponin-T antibody (Sigma Aldrich) diluted 1:30,000 in 5% NFDM and TBS-T. Following washing with TBS-T, blots were incubated for 1 h at room temperature with anti-rabbit (desmin) or anti-mouse (troponin-T) horseradish peroxidase (**HRP**; Cell Signaling Technology, Danvers, MA) diluted 1:20,000 in 5% NFDM and TBS-T was combined and were. Blots were developed using an enhanced chemiluminescence kit (ECL Plus; GE Healthcare Bio-Sciences) and imaged using a ChemiDoc-It 415 Imaging System (UVP). VisionWorksLS Image Acquisition and Analysis

Software (UVP) were used to measure band intensities. Degraded desmin-38 kDa, degraded desmin-35 kDa, and intact desmin were measured at 38 kDa, 35 kDa, and 55 kDa, respectively (Fig. 2b and 3b). Degraded and intact bands of troponin-T were measured at 30 and 40 kDa, respectively (Fig. 2c and 3c). Bands were equalized for each blot to a pooled sample.

Statistical Analysis

Steak data from the ST and LL were analyzed using a randomized split plot design with the PROC MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC) with a $5 \times 5 \times 4$, and a $3 \times 3 \times 4$ factorial arrangements, respectively. Postmortem day of aging (**DOA**), location (**LOC**), and dietary treatment (**TRT**), along with their interactions were fixed effects; quadratic and linear effects of DOA were analyzed for WBSF, sarcomere length, calpain activity, along with desmin and troponin degradation were analyzed. Steak LOC and TRT, along with their interactions were analyzed for fiber CSA and fiber type percentages. Differences for all data were considered significant at $P \leq 0.05$.

Results

Warner-Bratzler Shear Force

There were no 2- or 3-way interactions for ST or LL steaks' WBSF of ($P > 0.12$); therefore, only main effects will be presented.

As expected, there was a DOA effect (Fig. 5; quadratic, $P < 0.01$) for WBSF in the ST. Shear force values of the ST decreased between d 7 and d 28, as well as between d 28 and d 56 ($P < 0.01$). However, shear force in the ST was not different ($P = 0.08$) from d 7 to d 14, and also did not differ ($P = 0.43$) from d 14 and d 28. Shear force in the ST did not differ ($P = 0.67$) from d 56 to d 112. Semitendinosus steak LOC affected (Table 2; $P < 0.01$) WBSF values, with LOC 1 and LOC 2 not differing ($P = 0.87$). Both LOC 1 and LOC 2 had greater WBSF than LOC 3, 4,

and 5 ($P < 0.01$), and LOC 4 had the smallest WBSF value compared to all other LOC ($P \leq 0.02$). Steaks from LOC 3 and 5 did not differ ($P > 0.99$) in WBSF. Dietary TRT did not have an effect (Table 3; $P = 0.51$) on WBSF values in ST steaks.

Predictably, there was a DOA effect (Fig. 5; quadratic; $P < 0.01$) for LL steak WBSF. Shear force differed at each DOA ($P < 0.01$) and declined more between d 7 and d 28 ($P < 0.01$; slope = 0.02 kg/day), than between d 28 and d 112 ($P < 0.01$; slope = 0.01 kg/day). A steak LOC effect (Table 2; $P = 0.88$) did not exist for WBSF values in LL steaks. Dietary TRT had an effect (Table 3; $P < 0.01$) on LL steaks, as CON steaks presented increased ($P < 0.01$) WBSF values compared to the other TRT, which had similar ($P \geq 0.91$) WBSF values.

Sarcomere Length

There were no 3-way interactions for sarcomere length of ST or LL steaks ($P \geq 0.28$). There was a DOA \times LOC interaction (Fig. 6; $P < 0.01$) for the sarcomere length of the ST steaks. Generally, LOC 5 of the ST had longer sarcomeres compared to LOC 1, 2, and 3 ($P \leq 0.03$) across the DOA. However, there were no differences in LOC 5 compared to LOC 1 at d 56, along with no differences in LOC 5 compared to LOC 2 at d 14, and no differences in LOC 5 compared to LOC 3 at d 112 ($P \geq 0.07$). Additionally, on d 7 ST LOC 4 had longer ($P = 0.03$) sarcomeres than LOC 1. Generally, ST sarcomere lengths in each LOC were similar throughout the 112 d aging period; however, LOC 3 sarcomere length increased ($P < 0.01$;) after d 56. Moreover, there were no DOA \times TRT or LOC \times TRT interactions for sarcomere length of ST steaks ($P \geq 0.39$; results not presented). Lastly, dietary TRT effect ($P \geq 0.56$; data not presented) on sarcomere length did not exist in ST steaks.

There were no 2-way interactions for sarcomere length in the LL steaks ($P \geq 0.62$;) Postmortem DOA affected (Table 4; quadratic, $P = 0.02$) sarcomere length LL steaks, with

decreased length at d 28 compared to d 7 and d 112 ($P \leq 0.03$), with no difference ($P = 0.95$) between those days. Sarcomere length in the LL was not affected by steak LOC or dietary TRT ($P \geq 0.83$).

Muscle Fiber Type Distribution and Cross-Sectional Area

There were no LOC \times TRT interactions for type I, type IIA, or type IIX fiber type percentages or CSA in ST or LL steaks ($P \geq 0.06$).

Steak LOC affected ST steak type I, type IIA, and type IIX fiber types in ($P \leq 0.01$; Fig. 7a). Percentages of type I fibers were not different between ST steak LOC 1, 2, and 3 ($P \geq 0.24$) and LOC 4 and 5 ($P = 0.76$); however, LOC 1, 2, and 3 possessed fewer type I fibers than LOC 4 and 5 ($P < 0.01$). Semitendinosus steak LOC 3 had fewer type IIA fibers than LOC 1 and LOC 5 ($P \leq 0.01$), while the other four locations did not differ in type IIA fiber percentages ($P \geq 0.19$). Meanwhile, ST steak LOC 1, 2, and 3 type IIX percentages did not differ ($P \geq 0.18$), and LOC 1 and 2 type IIX percentages did not differ from LOC 4 ($P \leq 0.18$). Steak 3 had a greater percentage of type IIX fibers than LOC 4 and 5 ($P \leq 0.01$), which possessed non-differing ($P = 0.25$) type IIX percentages in ST steaks. Finally, ST steak LOC 5 possessed fewer type IIX fibers than LOC 1, 2, and 3 ($P \leq 0.01$).

Similar to fiber type percentages, LOC affected ST steak CSA for all three muscle fiber types ($P \leq 0.01$; Fig. 7b). Type I, IIA, and IIX CSA followed the same pattern, as LOC 1 had larger fibers than LOC 2 and 3 ($P < 0.01$). Fibers in LOC 2 and 3 possessed non differing ($P \geq 0.89$) CSAs in ST steaks. Fibers in ST steak LOC 2 and 3 were larger than fibers in LOC 4 and LOC 5 ($P \leq 0.01$). Type I and type IIA fibers in ST steak LOC 4 and 5 possessed non differing CSA ($P \leq 0.12$), however, LOC 4 type IIX fibers were larger than LOC 5 ($P = 0.03$). Lastly, TRT did not affect type I, IIA, or IIX fiber percentages or CSA (Table 3; $P \geq 0.22$).

Steak LOC did not affect type I, IIA, and IIX percentages or CSA in LL steaks ($P \geq 0.08$). Dietary TRT did not affect Type I or IIA fiber percentages or CSA in LL steaks (Table 3; $P \geq 0.06$); however, TRT affected fiber type percentages and CSA of Type IIX fibers in LL steaks ($P < 0.03$). The percentages of Type IIX fibers in the ST of CON, AOX, and LATE steaks were similar ($P \geq 0.15$), while Type IIX percentages were not different in CON, ALG, and AOX ($P \geq 0.23$). However, LATE steaks had a greater ($P = 0.02$) percentage of Type IIX fibers than ALG steaks. The CON and LATE steaks were not different ($P = 0.99$), as well as, AOX and ALG steaks had non differing CSA ($P = 0.37$). Control and LATE steaks had a greater fiber CSA than AOX and ALG steaks ($P \leq 0.03$).

Calpain Activity

There were no 3-way interactions for intact calpain-1, autolyzed calpain-1, intact calpain-2, and autolyzed calpain-2 in ST or LL steaks ($P \geq 0.25$). Additionally, there were no DOA \times LOC or DOA \times TRT interactions for intact calpain-1, autolyzed calpain-1, intact calpain-2, and autolyzed calpain-2 in ST or LL steaks ($P \geq 0.16$; data not shown).

There were no LOC \times TRT interactions for autolyzed calpain-1, intact calpain-2, autolyzed calpain-2 in the ST ($P \geq 0.68$). There was a LOC \times TRT interaction (Fig 8; $P < 0.01$) for ST steaks intact calpain-1; however, adjusted P -values did not show significant differences of different LOC throughout TRT. There were no quadratic or linear DOA effects on autolyzed calpain-1, or intact and autolyzed calpain-2 in ST steaks ($P \geq 0.41$); however, ST steak intact calpain-1 had a quadratic DOA effect (Fig. 10; $P < 0.01$). As the amount of intact calpain-1 decreased ($P \leq 0.03$) until d 28, ceasing at d 56. Steak LOC and dietary TRT did not affect autolyzed calpain-1, intact calpain-2, and autolyzed calpain-2 ($P \geq 0.23$).

There were no LOC \times TRT interactions for autolyzed calpain-1, intact calpain-2, and autolyzed calpain-2 ($P \geq 0.90$). Intact calpain-1 presented a LOC \times TRT interaction (Fig. 9; $P = 0.01$) in LL steaks, however, adjusted p -values did not show significant differences of LOC throughout dietary TRT. There were no quadratic or linear DOA effects on autolyzed calpain-1, or intact and autolyzed calpain-2 in LL steaks ($P \geq 0.47$; data not shown). There was a quadratic DOA effect (Fig. 10b; $P < 0.01$) on intact calpain-1 in LL steaks, as the amount of intact calpain-1 decreased from d 7 to d 112 ($P = 0.04$), intact calpain-1 ceased at d 112. Steak LOC and dietary TRT did not affect autolyzed calpain-1, intact calpain-2, and autolyzed calpain-2 ($P \geq 0.74$; data not shown).

Desmin and Troponin-T Degradation

There were no 3-way interactions for intact desmin, degraded desmin-38 kDa, degraded desmin-35 kDa intact troponin-T, or degraded troponin-T in ST or LL steaks ($P \geq 0.06$). Furthermore, there were no DOA \times LOC or DOA \times TRT interactions for intact desmin, degraded desmin-38 kDa, and degraded desmin-35 kDa in ST steaks ($P \geq 0.23$). Semitendinosus steaks had no LOC \times TRT interactions on degraded desmin-38 kDa and degraded desmin-35 kDa in the ST ($P \geq 0.88$); however, there was a LOC \times TRT interaction (Fig. 11; $P = 0.03$) on intact desmin. The ALG steaks showed increased ($P = 0.03$) levels of intact desmin in LOC 4 compared to LOC 3; meanwhile the other TRT throughout the other LOC were not different ($P \geq 0.15$) in ST steaks. There was no quadratic DOA effect (Fig. 12a; $P = 0.70$) observed on degraded desmin-35 kDa in ST steaks; however, there was a quadratic DOA effect on intact desmin and degraded desmin-38 kDa for ST steaks (Fig. 12a; $P \leq 0.02$). Relative band densities of intact desmin in ST steaks were not different at d 7, 14, and 28 ($P \geq 0.44$), before decreasing ($P < 0.01$) at d 56; the band densities of intact desmin were not different between d 56 and d 112

($P = 0.76$). Band intensities of degraded desmin-38 kDa in ST steaks were not different ($P = 0.87$) at d 7 and d 14, however band intensities increased ($P \leq 0.04$) at d 28 and did not differ ($P > 0.16$) throughout the rest of the aging period. There was a linear DOA effect ($P = 0.01$) on degraded desmin-35 kDa band densities in ST steaks. Degraded desmin-35 kDa band densities did not differ ($P \geq 0.30$) from d 7 to d 28, however, the band intensity of degraded desmin-35 kDa in ST steaks increased ($P = 0.03$) at d 56 compared to d 7, while d 56 had non-differing relative band densities compared to d 14 and d 28 ($P \geq 0.06$). The relative band density of degraded desmin-35 kDa was the greatest at d 112 of all the aging periods ($P < 0.01$) in ST steaks. There were no TRT or LOC effects on intact desmin, degraded desmin-35 kDa, or degraded desmin-38 kDa in ST steaks ($P \geq 0.13$; data not shown).

There were no 2-way interactions for intact desmin, degraded desmin-38 kDa, and degraded desmin-35 kDa in LL steaks ($P \geq 0.40$). No quadratic DOA effect existed on intact desmin and degraded desmin-35 kDa (Fig. 13a; $P \geq 0.15$). However, there was a quadratic DOA effect ($P < 0.01$) on degraded desmin-38 kDa in LL steaks, as band densities increased (Fig. 13a; $P = 0.05$) from d 7 to d 28 and decreased between d 28 and d 112 ($P = 0.01$). The relative band density of degraded desmin-38 kDa was non-differing ($P = 0.99$) in LL steaks on d 7 and d 112. There was a linear DOA effect for intact and degraded desmin-35 kDa (Fig. 13a; $P \leq 0.01$). Band densities for intact desmin in LL steaks were not different ($P = 0.92$) from d 7 to d 28, before decreasing ($P < 0.01$) at d 112. Meanwhile, relative band densities for degraded desmin-35 kDa in LL steaks were not different ($P = 0.92$) from d 7 to d 28, before decreasing ($P < 0.01$) at d 112. Finally, TRT and LOC did not affect the band densities of intact or degraded desmin-38 kDa or degraded desmin-35 kDa in LL steaks ($P \geq 0.13$).

There were no 2-way interactions for intact and degraded troponin-T in ST steaks ($P \geq 0.06$). A quadratic DOA effect for ST steaks was observed for intact and degraded troponin-T (Fig. 12b; $P \leq 0.05$). The band density of intact troponin-T in ST steaks did not differ ($P = 0.11$) between d 7 and d 14, however, band densities decreased ($P = 0.04$) at d 28. After d 28, the band densities of intact troponin-T were not different ($P \geq 0.67$) through d 112. Similarly to intact troponin-T of ST steaks, the band densities of degraded troponin-T in ST steaks were similar ($P = 0.11$) between d 7 and d 14, before increasing ($P \leq 0.01$) at d 28. After d 28, the band densities of degraded troponin-T in ST steaks were not different through d 112 ($P \geq 0.22$).

There was no LOC effect on relative band densities of intact or degraded troponin-T for ST steaks ($P \geq 0.34$). However, there was a TRT effect ($P = 0.01$) on the relative band densities of intact troponin-T. The unadjusted P -values ($P = 0.04$) showed the AOX to have a lower relative band density than that of the CON TRT, however, once P -values were adjusted, this effect was negligible.

There was no DOA \times LOC interaction ($P = 0.45$) for relative band densities of degraded troponin-T in LL steaks, however, there was a DOA \times LOC interaction (Appendix A; $P < 0.01$) for intact troponin-T. The adjusted P -values showed no differences in means across LOC throughout DOA. The relative band densities of degraded and troponin-T in LL steaks were not affected by the 2-way interactions DOA \times TRT, or LOC \times TRT ($P \geq 0.19$). A quadratic DOA effect (Fig. 13b; $P < 0.01$) was presented for degraded troponin-T, the relative band density of LL steaks increased from d 7 to d 28 (Fig. 13b; $P < 0.01$), then presented non-differing ($P = 0.87$) values at d 28 and d 112. Meanwhile, there was no quadratic DOA effect ($P = 0.15$), however intact troponin-T in LL steaks decreased linearly ($P < 0.01$) throughout the aging period. The relative band density decreased ($P = 0.02$) from d 7 to d 28, then decreased

($P < 0.01$) from d 28 to d 112. Longissimus lumborum steak LOC had no effect ($P = 0.68$) on degraded troponin-T. However, steak LOC did affect intact troponin-T ($P < 0.01$); the amount of intact troponin-T increased from LL steak LOC 1 to LOC 3 (Table 2; $P < 0.01$), while LOC 2 showed non-differing band densities to both LOC ($P \geq 0.68$). Dietary TRT did not impact intact or degraded troponin-T in LL steaks ($P \geq 0.40$).

Discussion

Consumers are willing to pay \$1.23/kg more for a product considered “guaranteed tender” (Miller et al., 2001). It can be said tenderness is one of the most important attributes consumers consider when purchasing meat. The 2010 National Beef Quality Audit reported beef was aged from 1 to 358 d for all retail aged retail beef cuts (Igo et al., 2001). However, research regarding extended postmortem aging is limited in the LL and ST, as the maximum length of time studied is 63 d for the LL (Karney et al., 2014) and 70 d for the ST (Phelps et al., 2016a), respectively.

Postmortem aging increases meat tenderness (Lehman, 1907; Moran and Smith, 1929; Gruber et al., 2006; Juárez et al., 2010); however, the effect of extended postmortem aging, past d 70 (Phelps et al., 2016a) has not been examined. The accepted WBSF value which consumers are able to distinguish a difference in tenderness is 1 kg (Miller et al., 1995). The aging response is highly dependent upon individual muscles (Gruber et al., 2014). Our results showed extended postmortem aging increased meat tenderness by 21.5% through d 56 in the ST. Furthermore, tenderness increased by 33.0% in the LL. In accordance to previous literature, the current study showed rapid increases in tenderness in the first 28 d of aging in both the ST and LL. Tenderness of ST steaks increased by 8.8% from d 7 to d 28. Additionally, WBSF values decreased by 15.0% in the LL from d 7 to d 28. Similarly, Gruber et al. (2006) reported a 17.4% decrease in

shear force values in the LL and a 19.8% decrease in shear force values in the ST between d 6 to d 28 postmortem, thereby producing a more tender product throughout the study's 28 d aging period. In the current study, ST steaks continued the increase in tenderness, as WBSF values decreased by 15% through d 56. This is a discrepancy from Juárez et al. (2010) which reports no tenderness improvement after d 28 during the 56 d aging period. The current study had decreased shear force values (4.39 kg) at d 56 compared to the 6.95 kg at d 56 in the Juárez et al. (2010) study. This decreased shear force could be attributed to the difference in cooking temperatures, as Juárez et al. (2010) cooked steaks to an internal temperature of 72°C, bagged, and placed in an ice bath to reduce the temperature rise, whereas, the steaks in the current study were cooked to 65°C and allowed to rise to a peak temperature of 71°C.

Aging periods in the LL were unequally spaced, as the first aging period consisted of 28 d and the second aging period of 84 d. During the first 28 d WBSF decreased by 0.6 kg during the first 21 d, compared to 0.7 kg in the last 84 d, a higher proportion of aging occurred by d 28. This is in accordance with a multitude of aging studies that suggest the largest gains in tenderness will be achieved by d 14 (Calkins and Seidman, 1988; Koohmaraie et al., 1991).

Meat tenderization has been attributed to various endogenous factors within the muscle, these include sarcomere length, muscle fiber type and CSA, as well as postmortem proteolysis and calpain activity. In the current study, sarcomere length decreased by 2% at d 28 compared to d 7 and d 112 values. The exact affect of postmortem aging on sarcomere length and the amount of sarcomere length variation which affects meat tenderness has not been determined. Bouton et al. (1973) measured sarcomere length in mutton across aging periods and determined 0.06 μm was a non-statistical and non-biological difference in sarcomere length, whereas the difference in sarcomere length of the current study was 0.04 μm . Starkey et al. (2015) measured sarcomere

length at 1, 7, and 14 d postmortem in lamb longissimus muscles. The authors reported no difference in sarcomere length throughout the 14 d aging period, sarcomere length varied 0.05 μm . Geesink (2001) also examined sarcomere length across aging periods in lamb and found no differences in sarcomere length. However, Geesink (2001) suggested the difference in sarcomere length across various aging times could be attributed to sampling procedure differences.

Tenderization is dependent on the net proteolysis from calpain activity and inactivation. The activity of calpain-1 and calpain-2 is highly dependent upon the amount of calcium released in the muscle (Dransfield, 1993), the amount of calcium which is required to activate calpain-1 (μm calcium) is considerably less than that which is required for calpain-2 (mm calcium). Therefore, as calpain-1 is activated with minimal amounts of calcium, the role calpain-1 plays in the tenderization of meat is substantially greater and is especially critical when examining postmortem tenderness (Dransfield, 1993; Dransfield 1994). Our results indicated intact calpain-1 decreased throughout the aging period in the ST and LL by 88.5% and 99.3%, respectively, and intact calpain-1 disappeared by 112 d in the ST or LL steaks. This study did not show a difference in autolyzed calpain-1, or autolyzed and intact calpain-2. Geesink and Koohmaraie (1999) were in agreement as they examined the calpain activity of lamb and reported similar results to the current study, as the intact calpain-1 value reduced to 94% of the d 0 activity. In addition, the lamb longissimus also produced a stable calpain-2 activity. The stability of calpain-2 throughout aging can be attributed to the insufficient levels of calcium required to activate calpain-2. Phelps et al. (2016a) showed a complete disappearance of intact calpain-1 by d 70 in the ST. Furthermore, Phelps et al. (2016) indicated continued decrease of intact calpain-2 through d 70 of aging, with intact calpain-2 and autolyzed calpain-1 activity still detected through d 70. The intact calpain-1 disappeared throughout the aging period due to the amount of

calcium required to activate the calcium. Our results showed no intact calpain-1 activity past d 56. Since the amount of calcium taken to autolyze calpain-1 is relatively low, autolysis of calpain-1 occurs fairly rapidly.

Postmortem proteolysis and calpain activity have been shown to have the greatest impact on postmortem aging. Desmin is critical in maintaining the structural component of muscle cells, as desmin is an intermediate filament which wraps around the z-line, therefore, the degradation of this filament is a direct factor of meat tenderness (Huff-Lonergan, et al., 2010). The desmin filament is known to degrade during postmortem aging (Huff-Lonergan, 1999; Melody et al., 2004) and degradation has been linked to the presence of calpain-1, as calpain-1 degrades desmin throughout aging. Degradation of the desmin filament occurs more rapidly in myofibrils with low shear force (Huff-Lonergan and Lonergan, 1999, Melody et al., 2004). The current study showed a quadratic effect of intact desmin, with a decrease of 17.5% from d 56 to d 112 in the ST, however the largest decrease (59.7%) in intact desmin occurred between d 28 and d 56. Furthermore, in the LL had an 82.4% decrease in intact desmin occur between d 28 and d 112. Huff-Lonergan et al. (1996) reported desmin degradation through d 56 in the beef LL, whereas, Phelps, et al. (2016) study also reported similar degradation in ST steaks. Degraded desmin was also present in two forms in this study-desmin – 38 kDa and desmin – 35 kDa. Desmin – 38 kDa is a major degradation product. This desmin degradation product which occurs at 38 kDa is commonly reported as degraded desmin (Huff-Lonergan et al., 1996; Lonergan 2001; Anderson et al., 2007). Huff-Lonergan et al. (1996) reported degraded desmin at 38 kDa was present until d 56, at which point a secondary band of degraded desmin appeared at d 56. Similarly to the Huff-Lonergan study, the degraded desmin at 38 kDa continued degrading to the 35 kDa band. In the current study, degraded desmin at 38 kDa appeared at d 7 postmortem. Desmin – 38 kDa

appeared at 7 d postmortem in this study and continued to increase through d 28. After d 28, the degraded desmin began to shift to the 35 kDa band. Desmin – 35 kDa increased in a quadratic fashion through d 112, and by d 112 the amount of degraded desmin – 35 kDa was greater than that of degraded desmin – 38 kDa. The band at 35 – kDa most commonly appears after meat has aged over an extended period of time. The role of desmin in explaining tenderness variation varies throughout different studies. Most closely related to the current study, Rhee et al. (2004) examined tenderness within the LL and concluded there was a high correlation of beef tenderness and desmin degradation (-0.62). Whereas Starkey et al. (2015) examined desmin degradation in the lamb longissimus and concluded 12.7% of tenderness variation occurred to desmin degradation. This difference in correlation and contribution of desmin degradation to meat tenderness can be attributed to species differences.

While troponin-T has not been regarded as the cause of tenderization, it has been deduced as a key indicator that tenderization has occurred. Troponin-T is a non-structural protein which can be degraded by calpain-1 (Huff-Lonergan et al., 2010) and is often associated with the tenderness of beef (MacBride and Parrish, 1977; Huff-Lonergan et al., 2010). The current study's results showed the amount of intact troponin-T decreased in a quadratic fashion in the ST, as the relative band density of intact troponin-T decreased to 45.0% of the d 7 value by d 28 and 20.0% of the initial value by d 112. The relative band density of intact troponin-T in the LL was 63.3% by d 28 and 21.2% by d 112 of the initial value. The ST's degraded troponin increased most rapidly between d 7 and d 28. Degraded troponin-T in the LL followed suit to the ST, as the amount of degraded troponin-T rapidly increased before d 28, then leveling off between d 28 and d 112. Previous literature supports the continued degradation of desmin and troponin through extended postmortem aging. Phelps et al. (2016a) reported desmin and

troponin-T degradation continued through d 70 in ST steaks, while Huff-Lonergan (2010) also reported continued degradation through d 56 in LL steaks.

The second portion of this study examined a tenderness gradient throughout the ST and LL. A well-established intramuscular tenderness gradient in various muscles has been reported (Shackelford et al., 1997; Reuter et al., 2002; Rhee et al., 2004; Janz et al., 2006; Derrington et al., 2010; Senaratne et al., 2010; Phelps et al., 2016a) which has led to important marketing decisions of muscles throughout the beef carcass. Results in this particular study were in agreement to previous literature, in which an intramuscular tenderness gradient in the ST was apparent. The mid (LOC 3), mid-distal (LOC 4), and distal (LOC 5) locations were the most tender portions of the muscle, with the mid-distal portion presenting the absolute lowest shear force value. Phelps et al. (2016) reported the lowest shear force values in steaks 7 and 8, which corresponds to LOC 4 in the current study. Tenderness throughout the entire ST decreased 11.25% from the most proximal portion to the most distal portion of the muscle. The lowest shear force value occurred in the mid-distal portion, which had decreased WBSF values of 17.5% compared to the most proximal portion (LOC 1) of the ST. In contrast, Reuter et al., (2002) reported the lowest WBSF in the middle portion of the ST, which corresponds to LOC 3 in this particular study. Furthermore, Shackelford et al. (1997) reported a tenderness gradient within the ST, in which the most proximal portion of the muscle presented the highest shear force values compared to the most distal portion of the ST, which had the lowest ST values. The current study solidified results of the previous studies, as the proximal portion of the ST was the least tender portion of the muscle with the most tender location occurring at the mid-distal region of the ST. A tenderness gradient was not noticeable in the LL, partially due to the locations within the LL, as only the six most posterior steaks of the longissimus muscle were utilized.

Sarcomere length has an impact factor on cooked meat tenderness (Bouton et al., 1973; Asghar et al., 1978; Wheeler and Koohmaraie, 1994; Koohmaraie et al., 1996). Sarcomere length is the basic unit of muscle which is measured from z-line to z-line (Aberle et al., 2001) and shortens during rigor mortis (Koohmaraie et al., 1996). The sarcomere length of a muscle is affected by carcass suspension and due to the location of the ST within a carcass, the steak location within the ST impacted sarcomere length. The most distal portion of the ST (LOC 5) presented a longer sarcomere compared to the other locations which mirrors the tenderness data, as the mid, mid-distal, and distal portions of the muscle had WBSF values below the USDA tenderness threshold. Sarcomere length $> 2.0 \mu\text{m}$ has been accompanied by increased tenderness (Bouton et al., 1973; Wheeler et al., 2000; Rhee et al., 2004); however, no differences in sarcomere length across the LL were detected. The sarcomere length data is a direct reflection of the WBSF values in both the ST and LL. In the ST the sarcomeres were longer and steaks were more tender in the mid distal/distal portions of the muscle throughout the aging period, compared to the most proximal portion of the muscle. In the LL steaks there was no difference in sarcomere length or WBSF values in any steak location.

Dransfield et al. (2003) argued muscle fiber type played a larger role in beef tenderness than CSA. Type I fibers, which are oxidative in nature and are generally more abundant in muscles generally classified as “tender” muscles. There was a 36% increase in Type I fibers and a 13.6% decrease in Type IIX muscle fibers from the most proximal to distal location of the ST. Type IIX fibers have the largest CSA of the three muscle fiber types, and the mid-distal and distal portions presented the smallest CSA in the study, along with the lowest percentages of Type IIX fibers. This led to the mid-distal and the distal locations being the most tender steak locations throughout the study. Calpain and proteolytic activity did not play a significant role in

the tenderness gradient of either the ST or LL. Phelps et al. (2016a) produced similar results as there was no location differences in proteolytic or calpain activity throughout the ST muscle.

Cross-sectional area is closely related to sarcomere length. Crouse et al. (1991) and Chriki et al. (2012) reported a greater CSA leading to a decrease in tenderness in beef muscles. The CSA decreased from the proximal (LOC 1) to distal (LOC 5) portion of the ST, with a 42% and 40% decrease in Type I and Type IIA muscle fibers, and Type IIX muscle fibers, respectively. This is in accordance to the Phelps (2016) study which examined the most proximal and distal portion of the ST and reported a decrease in CSA in all three fiber types from the proximal to distal portion of the ST. The current study was able to capture the gradient throughout each LOC of the ST, as fiber type and CSA were measured at each LOC of the ST. Ishii et al. (1995) measured the fiber type and CSA in the proximal, center, and distal portions of the ST in Holstein steers. The authors reported a decrease in the CSA of the middle portion of the ST and LL compared to the distal and proximal locations, overall, the muscle fiber CSA was larger than reported in Ishii et al. (1995). These differences could be attributed to the sample size or breed differences within each study. The current study examined 40 animals compared to the 4 animals studied in the Ishii et al. (1995) study. Additionally, the current study looked at feedlot steers whereas Ishii et al. (1995) utilized Holstein steers, which have been reported to have an increased amount of type I fibers compared to other larger breeds of cattle such as the German Angus (Albrecht et al., 2013). This can be attributed to the amount of type I fibers present in the muscle, as type I fibers are smaller due to their oxidative nature. The current study however, did not report a location difference in muscle fiber type in the LL for the 6 most posterior steaks.

A third aspect of this study was to examine the effect diets enhanced with algae had on meat tenderness. As consumer trends tend to lean toward consumption of less saturated fats and a

greater amount of polyunsaturated fatty acids, the meat industry is exploring feeding alternatives to improve the fatty acid profile of red meat. The effect of algae has been examined on meat quality, however, tenderness effects throughout postmortem aging have not been examined in the ST or LL. The effect of diet treatment was minimal throughout the study and mainly found in the LL. However, the microalgae treatment was more tender in the LL compared to the control diet. The extent of this effect is negligible, as there was a 0.48 kg decrease in WBSF values compared to the control, late fed algae, and the microalgae with antioxidants diets. Miller et al. (2001) established the threshold in which consumers are able to detect a difference in shear force values is 1.0 kg. Therefore, consumer detectable tenderness differences of the LL steaks were not detected across treatments. Phelps et al. (2016a) conducted a trained taste panel of ground beef processed with meat from heifers with diets supplemented with microalgae meal. The authors did not report a difference in tenderness values of ground beef processed from heifers with dietary supplementation.

Interestingly, the steaks from animals fed the Algae Diet throughout the feeding, along with the Algae and Added Antioxidant Diets presented the largest CSA in oxidative Type IIA fibers and glycolytic Type IIX fibers. This suggests a growth promotant factor in the microalgae fed treatment group, as the group fed microalgae in the final 10 d of finishing presented numerically higher CSA than those fed the control diet. This is not supported by tenderness values, however, as the treatment with the largest increase in CSA showed the lowest WBSF in the LL by 13.4%. The cross-sectional area of the ALG steaks do not support the well-established consensus that larger muscle fibers are associated with increased shear force values (Herring et al., 1965; Crouse et al., 1991; Renand et al., 2001). Therefore, there must be an underlying factor not in the scope of this study contributing to this tenderness difference. Connective tissue could

better explain the differences in these steak, as it is a major factor which influences tenderness, however, it was not included in the scope of this study. Further research is necessary to assess if the inclusion of algae for 45 d without antioxidants produces an increased amount of collagen in muscles.

According to Torrescano et al., (2003) connective tissue plays a critical role in meat tenderness. Furthermore, it is not just the amount of total collagen which plays a critical role, but also the amount of soluble and insoluble collagen that is present (Lepetit, 2007). The amount of soluble and insoluble collagen and the relationship to meat toughness is worthy of exploration in the extended postmortem aging time period and across different steak locations.

Conclusion

Various postmortem aging factors affecting tenderness were measured and their effect on meat tenderness is reported. Factors contributing to tenderness vary in postmortem aging, steak location, and dietary treatment effects. Tenderness differences throughout the postmortem aging period can be attributed to the postmortem proteolytic activity and calpain activity in the ST. The tenderness advances that occurred in the ST and LL throughout the aging period can be attributed to the concentration of intact calpain-1 in the muscle, as well as, the proteolytic breakdown of desmin and troponin. Whereas tenderness variation in steak locations may be attributed to differing sarcomere lengths, muscle fiber type, or CSA. This intramuscular tenderness gradient was best demonstrated within the ST, while a tenderness gradient did not appear in the LL, the inclusion of the entire LL will provide more conclusive results of the gradient within the longissimus muscle. Feeding algae to cattle produced a dietary treatment effect. However, the effect on beef tenderness was negligible as resulting tenderness values were below consumer detection thresholds. Therefore, meat tenderness does not benefit from algae

based diets. Tenderness stayed consistent from d 56 to 112 in the ST and continued to increase through 112 d postmortem in the LL. Tenderness gains are observed past reported average aging times, however, the gains in tenderness should be carefully considered. Further research on lower-valued muscle's, such as the ST, intramuscular tenderness gradient and response to extended postmortem aging is warranted in order to deliver the maximum eating experience to consumers choosing to consume beef steaks on a budget.

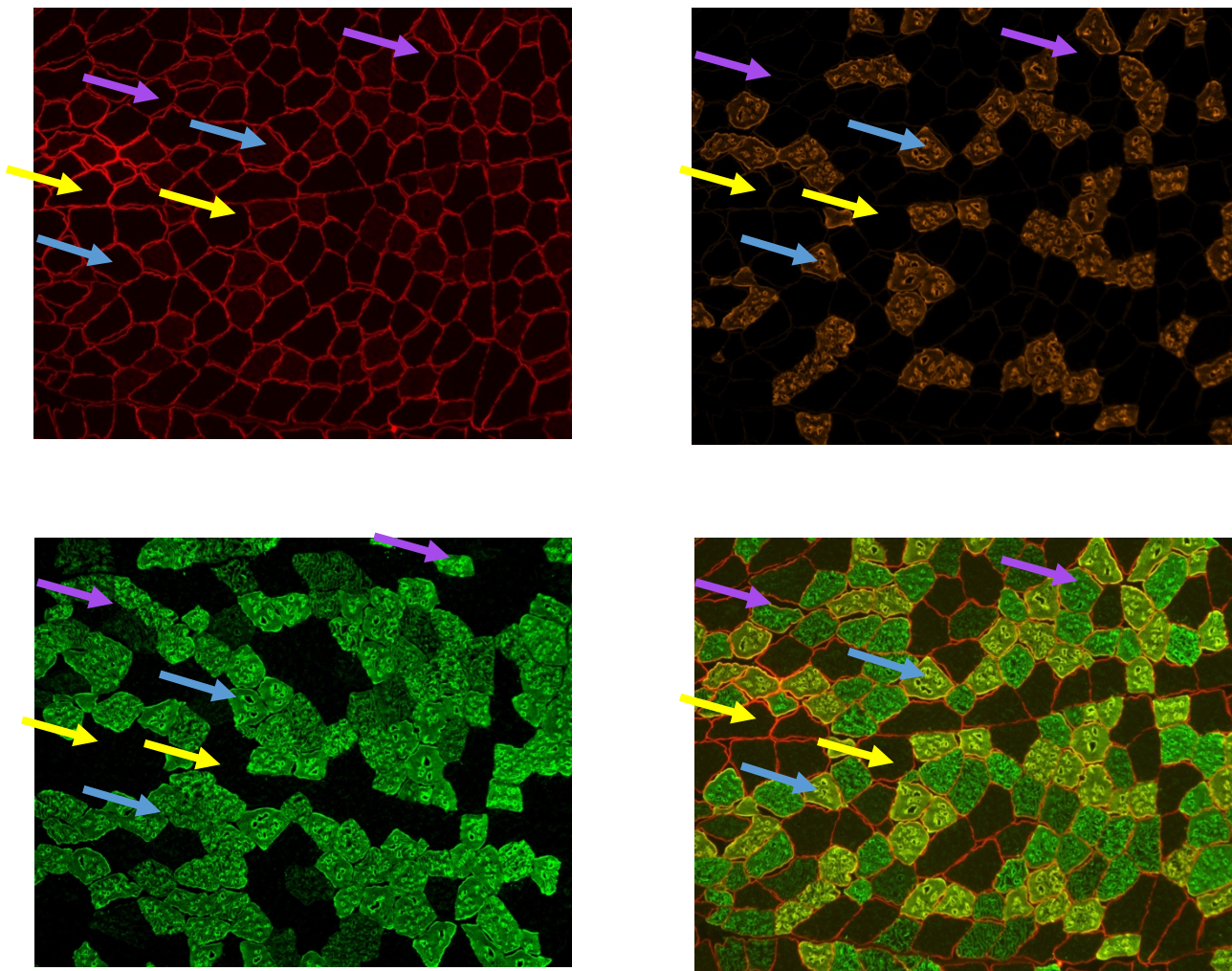


Figure 2. Representative photomicrographs of immunohistological staining pattern of beef Longissimus lumborum and Semitendinosus muscles. Fibers that stained positive for the BA-D5 antibody were categorized as type I fibers (Blue arrows). Fibers that stained positive for BF-35, but were negative for BA-D5 were categorized as type IIA fibers (Purple arrows). All fibers that were negative for the BF-35 antibody were categorized as type IIX fibers (Yellow arrows; Moreno- Sanchez et al., 2008; Schiaffino et al., 1989).

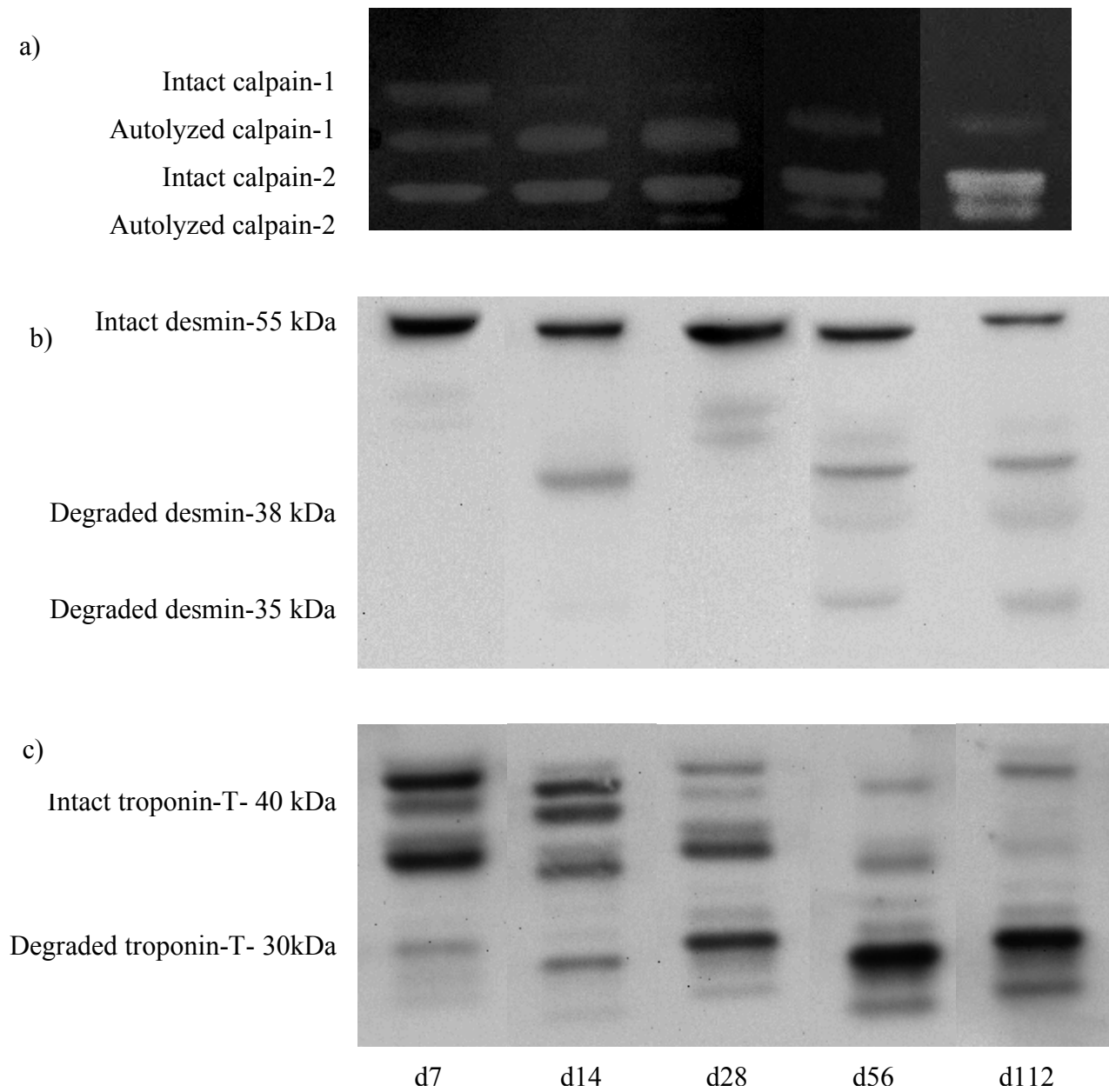


Figure 3. Representative a) calpain zymogram images, b) desmin immunoblot, and c) troponin-T immunoblot. Images encompass one steer's Semitendinosus steaks for 7, 14, 28, 56, or 112 d postmortem.

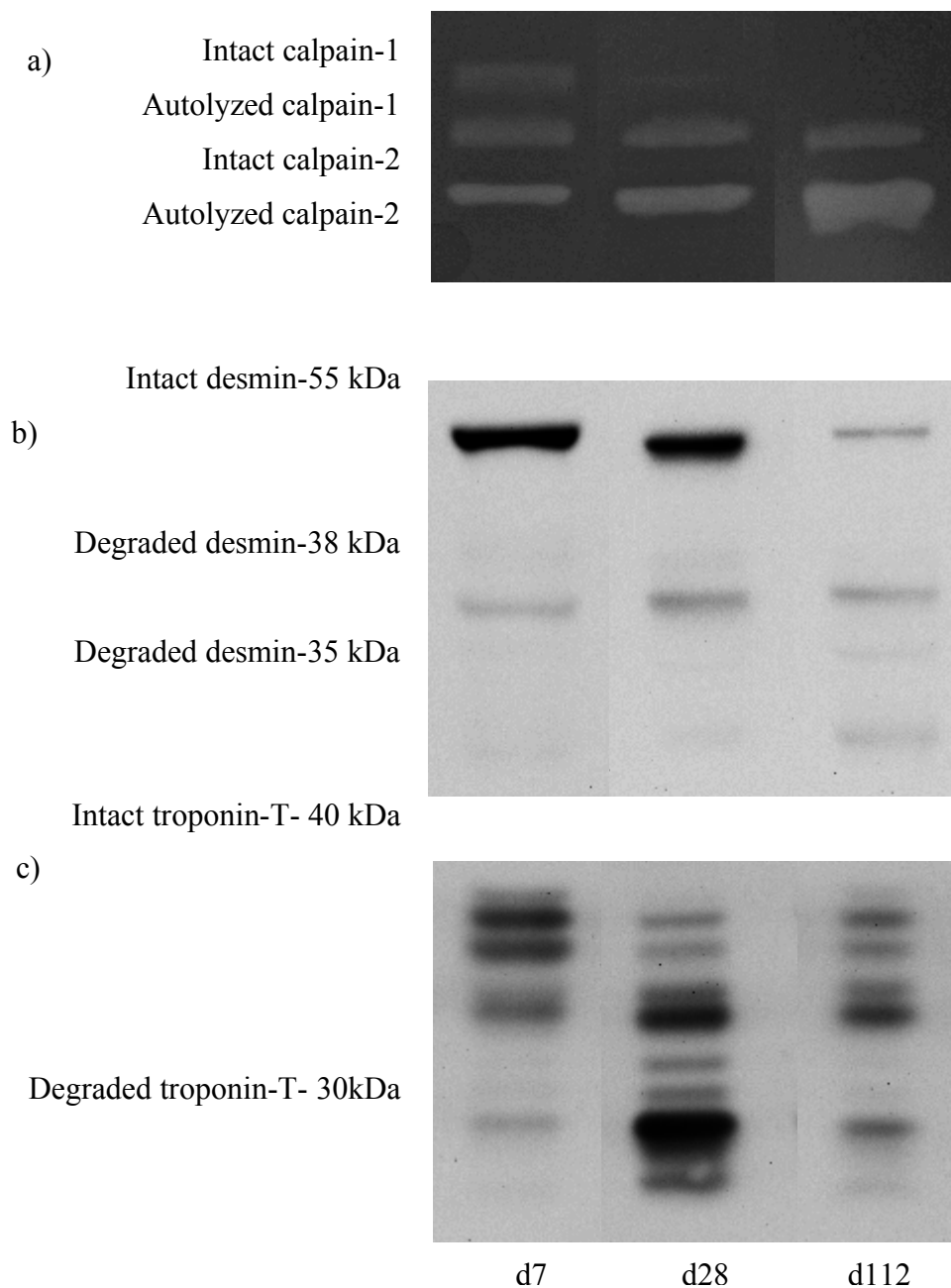


Figure 4. Representative a) calpain zymogram images, b) desmin immunoblot, and c) troponin-T immunoblot. Images encompass one steer's Longissimus lumborum steaks aged for 7, 28, or 112 d.

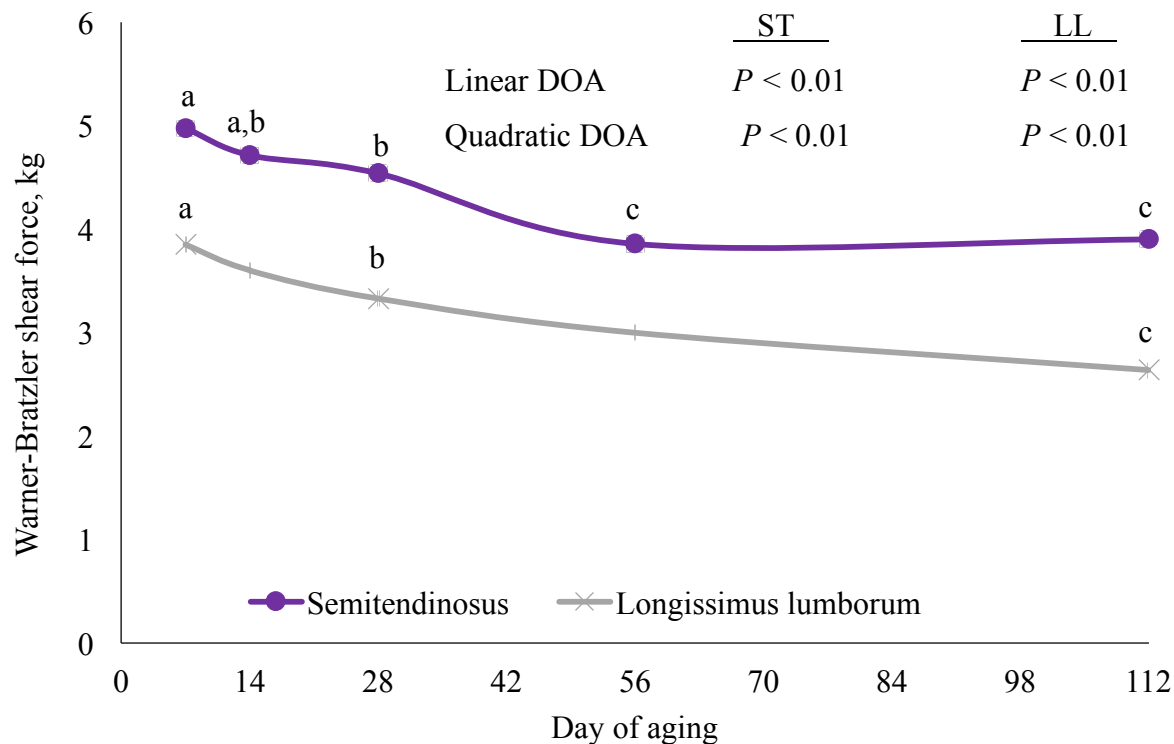


Figure 5. Effect of postmortem aging on Warner-Bratzler shear force of Semitendinosus (ST) steaks and Longissimus lumborum (LL). Steaks from ST were aged 7, 14, 28, 56, or 112 d postmortem. Steaks from LL were aged 7, 28, or 112 d postmortem. ^{a,b,c}Means within muscles are different ($P < 0.05$). SEM = 0.08.

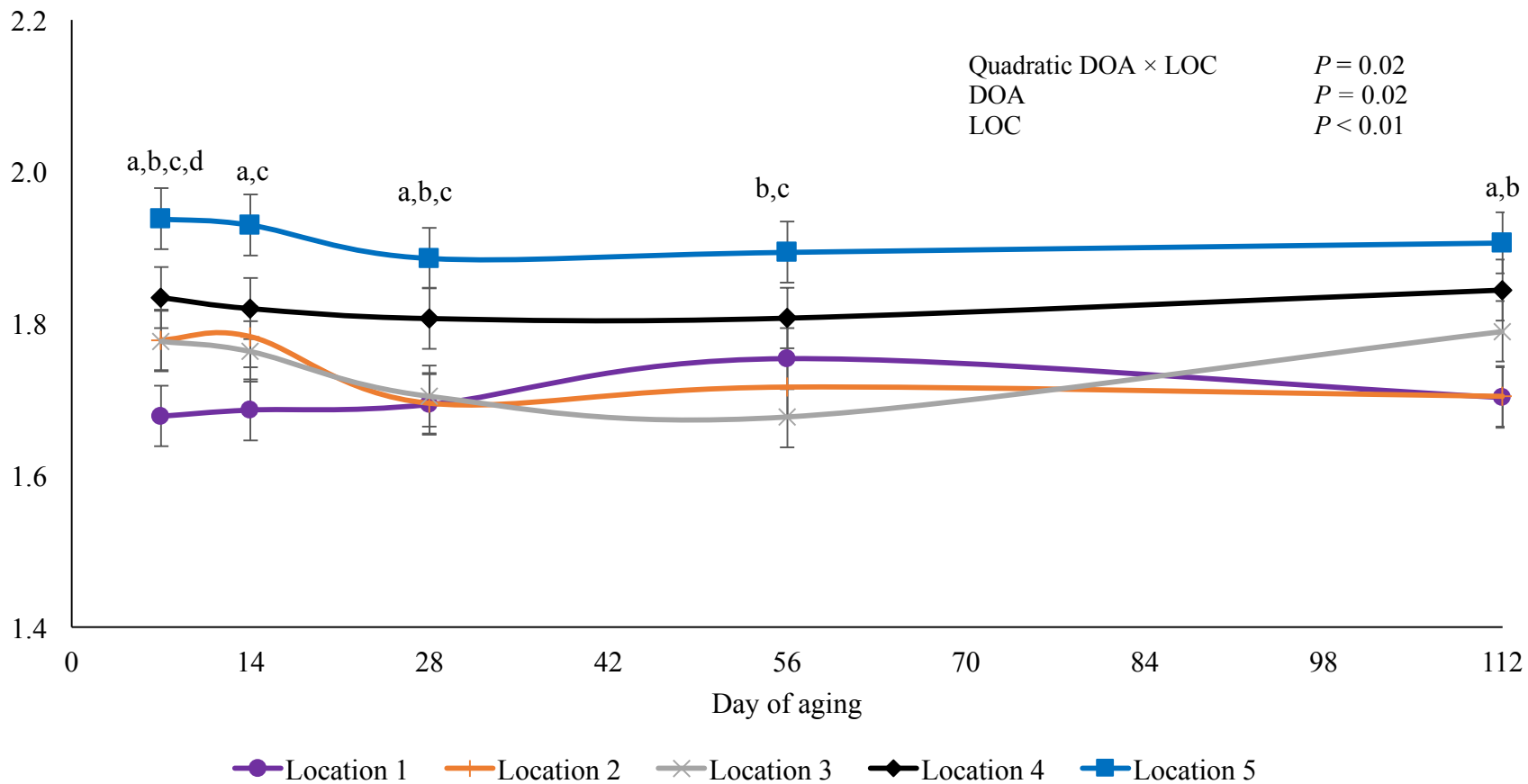


Figure 6. Interaction of postmortem aging (DOA) and steak location (LOC) on sarcomere length of Semitendinosus steaks (ST). The ST was fabricated into 10, 2.54-cm steaks, steak 1 being most proximal and steak 10 being most distal to the steers' body. Semitendinosus steaks were paired (1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10) and pairs were randomly assigned to 7, 14, 28, 56, and 112 d of aging. ^aMeans of LOC 5 are different ($P < 0.05$) from LOC 1 within an aging period. ^bMeans of LOC 5 are different ($P < 0.05$) from LOC 2 within an aging period. ^cMeans of LOC 5 are different ($P < 0.05$) from LOC 3 within an aging period. ^dMeans of LOC 4 are different ($P < 0.05$) from LOC 1 within an aging period. There were no differences between LOC 1, 2, and 3 in any of the aging periods.

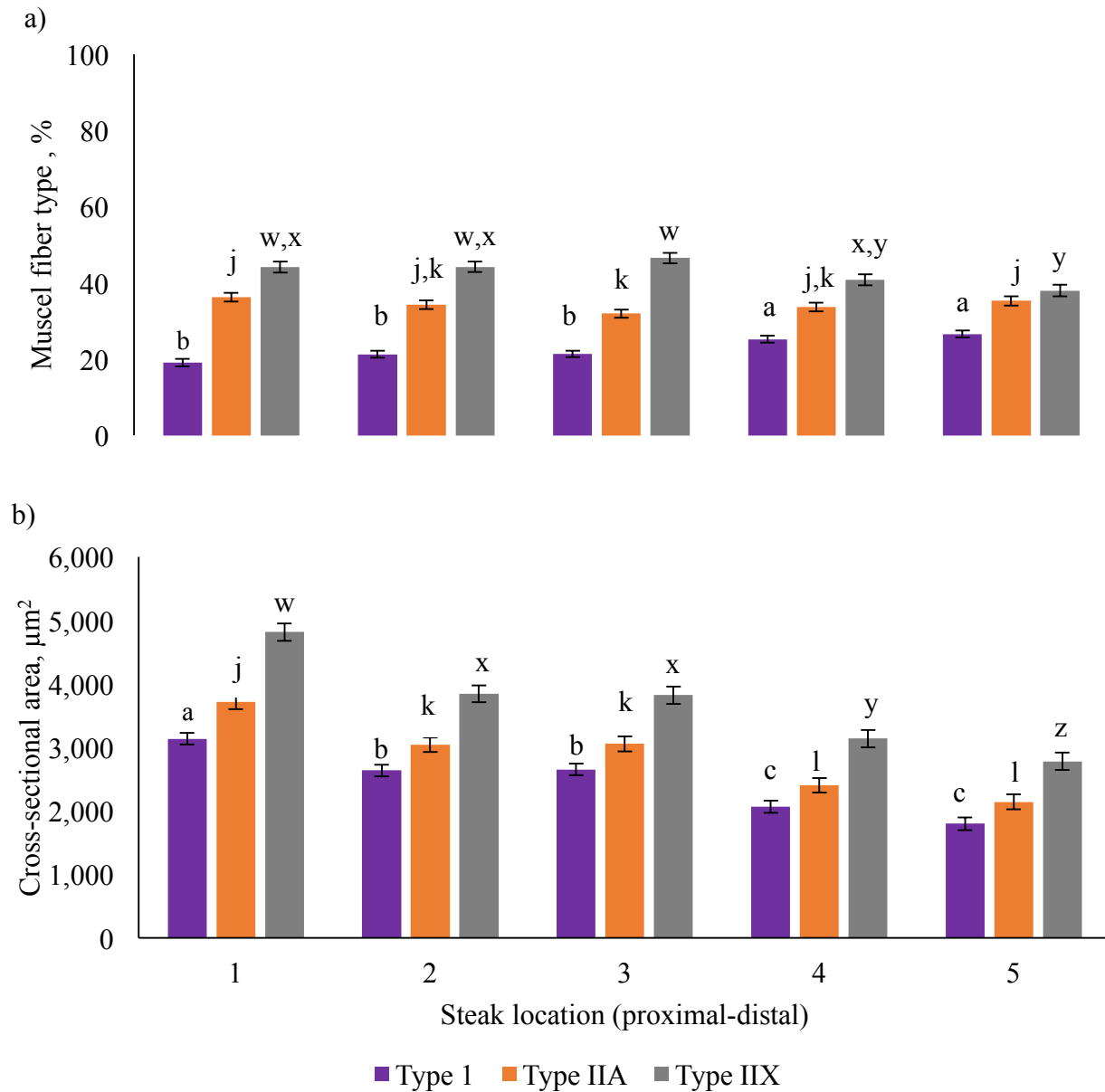


Figure 7. Effect of steak location on **a)** muscle fiber type percentage and **b)** muscle fiber cross sectional area of Semitendinosus (ST) steaks. The ST was fabricated into 10, 2.54-cm steaks, steak 1 being most proximal and steak 10 being most distal to the steers' body. Semitendinosus steaks were paired (1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10) and pairs were randomly assigned to 7, 14, 28, 56, and 112 d of aging. Steaks 1 and 2 comprised LOC 1, steaks 3 and 4 comprised LOC 2, steaks 5 and 6 comprised LOC 3, steaks 7 and 8 comprised LOC 4, and steaks 9 and 10 comprised LOC 5. ^{a,b,c}Means with different superscripts for Type I are different ($P < 0.05$). ^{j,k,l,m}Means with different superscripts for Type IIA are different ($P < 0.05$). ^{w,x}Means with different superscripts for Type IIX are different ($P < 0.05$). Steak LOC had an effect on muscle fiber type percentage and muscle fiber cross sectional area on Type I, Type IIA, and Type IIX ($P < 0.01$).

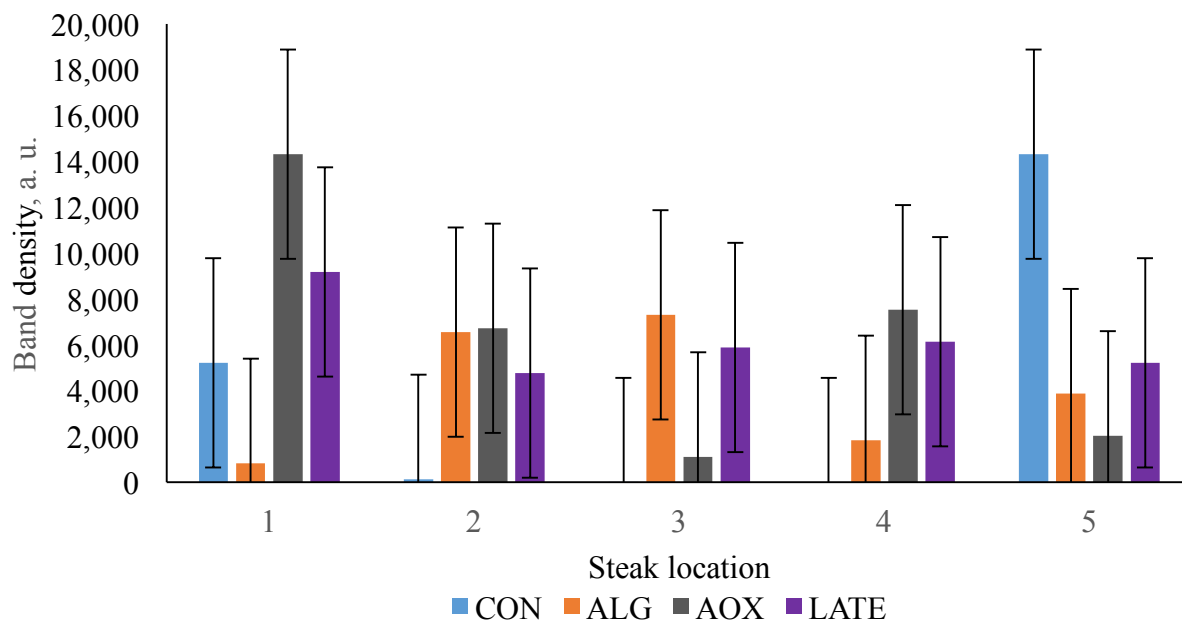


Figure 8. Effect of steak location and dietary treatment interaction on intact calpain-1 o Semitendinosus (ST) steaks. The ST was fabricated into 10, 2.54-cm steaks, steak 1 being most proximal and steak 10 being most distal to the steers' body. Semitendinosus steaks were paired (1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10) and pairs were randomly assigned to 7, 14, 28, 56, and 112 d of aging. Steaks 1 and 2 comprised LOC 1, steaks 3 and 4 comprised LOC 2, steaks 5 and 6 comprised LOC 3, steaks 7 and 8 comprised LOC 4, and steaks 9 and 10 comprised LOC 5.

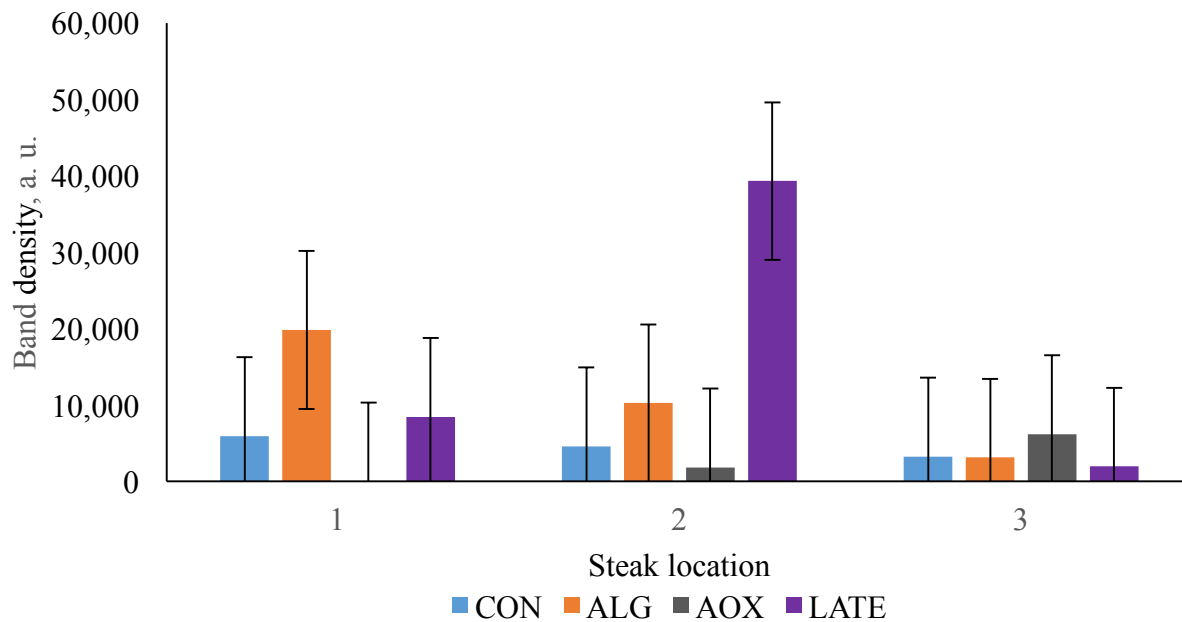


Figure 9. Effect of steak location and dietary treatment interaction on intact calpain-1 in Longissimus lumborum (LL) steaks. The LL was fabricated into 10, 2.54-cm steaks, steak 1 being most anterior and steak 6 being most posterior to the steers' body. Longissimus lumborum steaks were fabricated into 6-2.54 cm steaks and paired (1 and 2, 3 and 4, 5 and 6). Pairs were randomly assigned to 7, 28, and 112 d of aging. *Means are different ($P < 0.05$) within dietary treatment. Steaks 1 and 2 comprised LOC 1, steaks 3 and 4 comprised LOC 2,

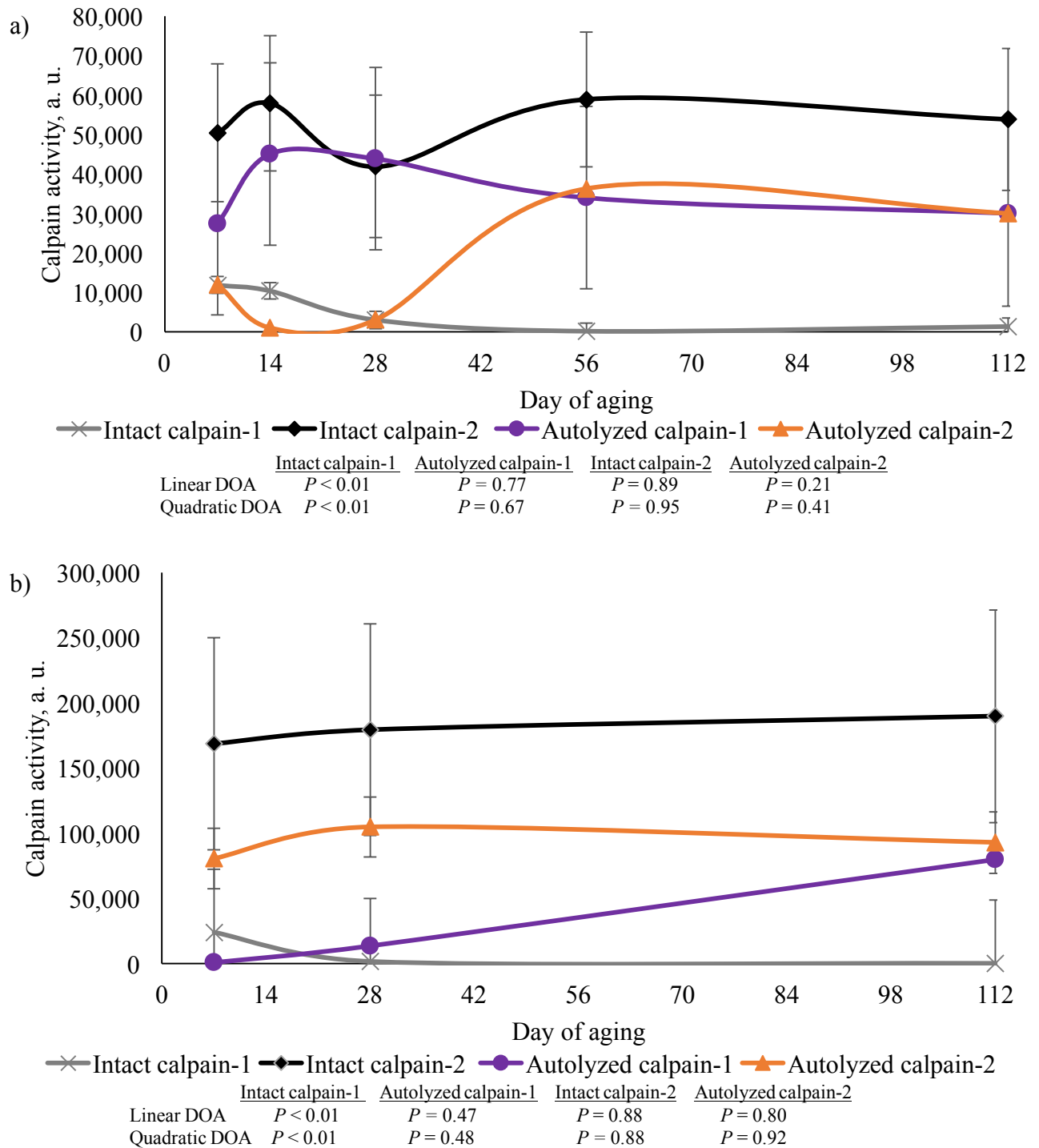


Figure 10. Effect of intact calpain-1, autolyzed calpain-1, intact calpain-2, and autolyzed calpain-2 of a) Semitendinosus steaks and b) Longissimus lumborum steaks. Semitendinosus steaks were aged for 7, 14, 28, 56, or 112 d postmortem. Longissimus lumborum steaks were aged for 7, 28, or 112 d postmortem.

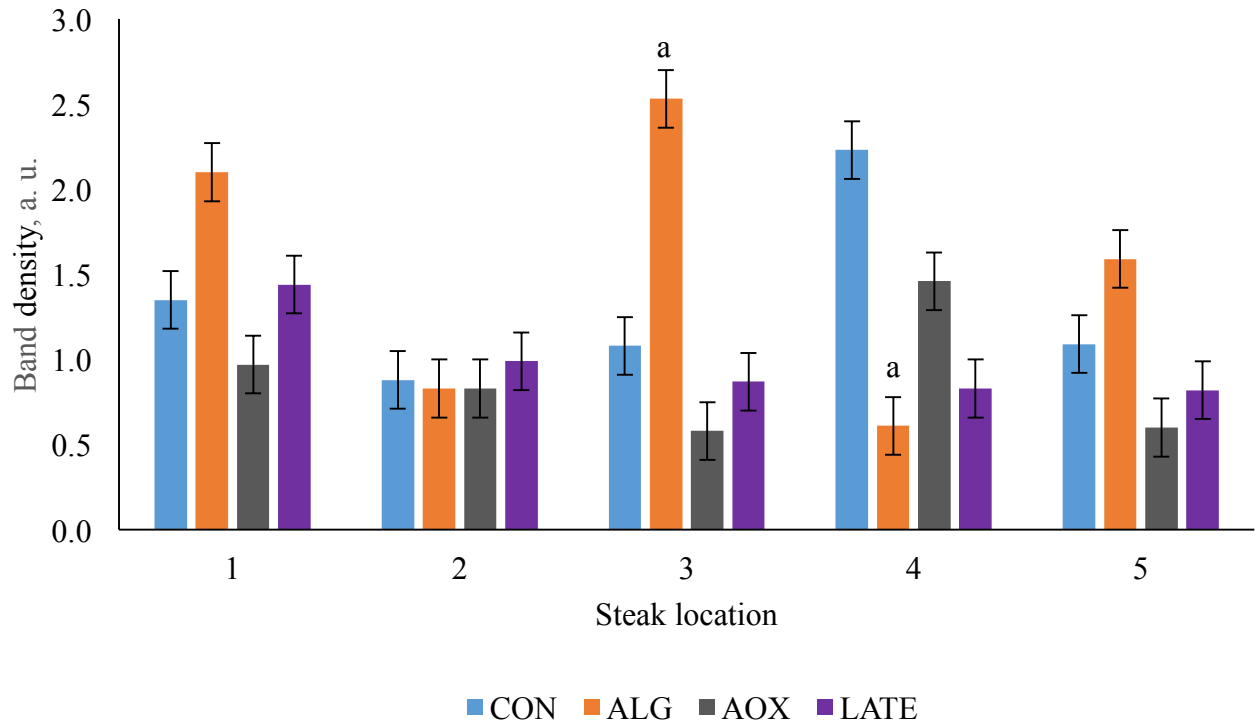


Figure 11. Steak location and dietary treatment interaction for intact troponin-T in Semitendinosus (ST) steaks. The ST was fabricated into 10, 2.54-cm steaks, steak 1 being most proximal and steak 10 being most distal to the steers' body. Semitendinosus steaks were paired (1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10) and pairs were randomly assigned to 7, 14, 28, 56, and 112 d of aging. ^aMeans are different ($P < 0.05$) within dietary treatment.

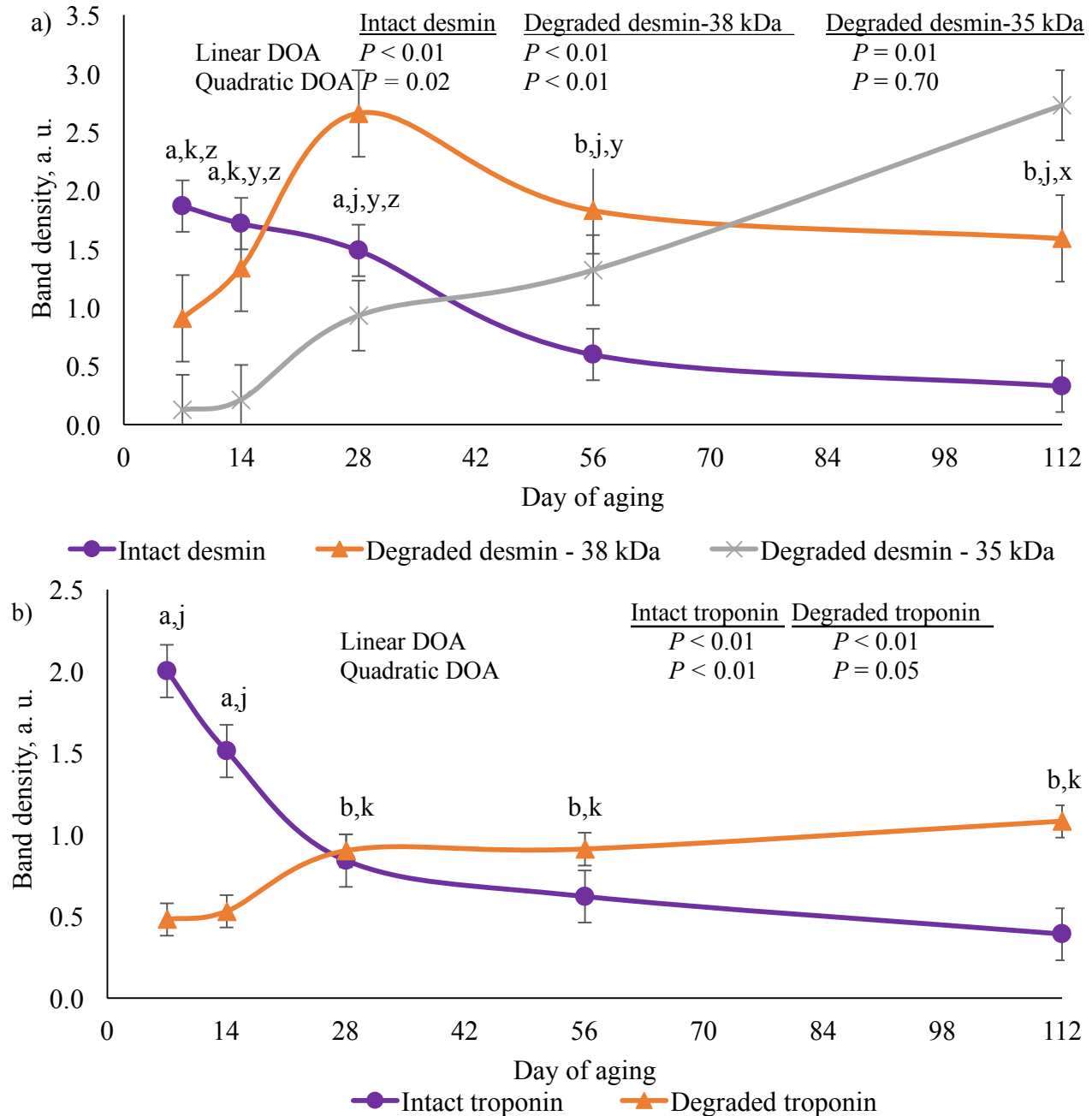


Figure 12. a) Intact, degraded desmin-38 kDa, and degraded desmin-35 kDa and b) intact and degraded troponin values throughout postmortem aging in Semitendinosus (ST) steaks. Steaks were aged 7, 14, 28, 56, and 112 d postmortem at 2°C. Immunoreactive bands located at 55 kDa, 38 kDa, and 35 kDa were identified as the intact, degraded desmin- 38 kDa, and degraded desmin 35- kDa, respectively. Immunoreactive bands located at 40 and 30 kDa were identified as intact and degraded forms of troponin-T, respectively. Bands were equalized to a pooled sample on each blot. ^{a,b}Means with different superscripts for intact desmin and troponin are different ($P < 0.05$). ^{j,k}Means with different superscripts for degraded desmin – 38 kDa and degraded troponin are different ($P < 0.05$). ^{x,y,z}Means with different superscripts for degraded desmin – 35 kDa are different ($P < 0.05$).

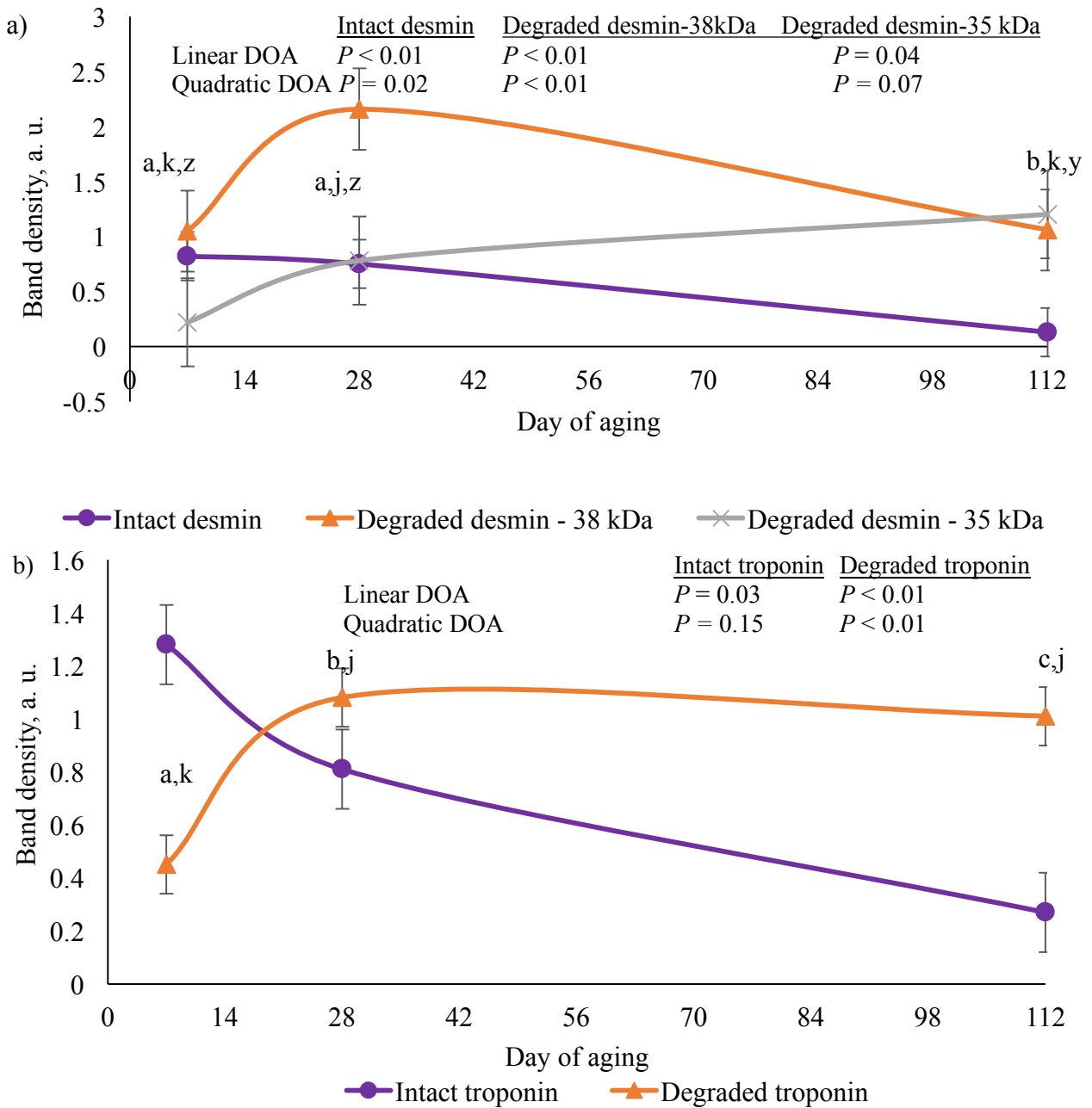


Figure 13. a) Intact, degraded desmin-38 kDa, and degraded desmin-35 kDa and **b)** intact and degraded troponin values throughout postmortem aging in Longissimus lumborum (LL) steaks aged 7, 28, and 112 d postmortem at 2°C. Immunoreactive bands located at 55 kDa, 38 kDa, and 35 kDa were identified as the intact, degraded desmin- 38 kDa, and degraded desmin 35- kDa, respectively. Immunoreactive bands located at 40 and 30 kDa were identified as intact and degraded forms of troponin-T, respectively. Bands were equalized to a pooled sample on each blot. ^{a,b}Means with different superscripts for intact desmin and troponin ($P < 0.05$). ^{j,k}Means with different superscripts for degraded desmin – 38 kDa and degraded troponin are different ($P < 0.05$). ^{x,y,z}Means with different superscripts for degraded desmin – 35 kDa are different ($P < 0.05$).

Table 1. Diets of steers fed control diet (**CON**), control diet plus 100 g·steer⁻¹·d⁻¹ microalgae meal (Alltech Inc., Nicholasville, KY) with antioxidants (103 IU/d vitamin E and Sel-Plex; Alltech Inc.) fed for the last 10 d of feeding (**LATE**), control diet plus 100 g·steer⁻¹·d⁻¹ microalgae meal fed during the finishing period (**ALG**), and 100 g·steer⁻¹·d⁻¹ microalgae meal plus antioxidants fed during the entire feeding period (**AOX**).

Ingredient, %	Treatment			
	CON	LATE	ALG	AOX
Steam-flaked corn	46.72	45.91	45.97	45.91
Wet-corn gluten feed	30.00	30.00	30.00	30.00
Corn silage	10.00	10.00	10.00	10.00
Ground flax	10.00	10.00	10.00	10.00
Monensin and tylosin premix ¹	1.35	1.35	1.35	1.35
Mineral/vitamin supplement ²	0.05	-	0.05	-
Mineral/vitamin supplement-no selenium selenite ³	-	0.01	-	0.01
Microalgae meal supplement ⁴	-	0.69	0.75	0.69
Ground limestone	1.59	1.59	1.59	1.59
Salt	0.25	0.25	0.25	0.25
Vitamin A ⁵ , 30,000 IU/g	0.007	0.007	0.007	0.007
Vitamin E ⁵ , 44,092 IU/g	0.03	0.14	0.03	0.14

¹Diet contained 300 mg·steer⁻¹·d⁻¹ monensin and 90 mg·steer⁻¹·d⁻¹ of tylosin (Elanco Animal Health, Greenfield, IN) provided to steers in a ground corn mixture.

²Diets consisted of CuSO₄, CoCO₃, ethylenediamine dihydriodide, MnSO₄, Na₂SeO₃, and ZnSO₄.

³Diets consisted of CuSO₄, CoCO₃, ethylenediamine dihydriodide, MnSO₄, and ZnSO₄, and organic yeast (Sel-Plex, Alltech Inc., Nicholasville, KY).

⁴Microalgae (*Schizochytrium limacinum*) fed to 100 mg·steer⁻¹·d⁻¹ level.

⁵Vitamin A was added at 2,205 IU/kg in all diets, CON and ALG diets included 22 IU/kg of Vitamin E, and AOX and LATE diets included 100 IU/kg.

Table 2. Main effect of steak location (**LOC**) on Warner-Bratzler shear force (**WBSF**) and intact and degraded troponin-T in the Semitendinosus (**ST**) and Longissimus lumborum (**LL**).

	Location ^{1,2}					SEM	P-value
	1	2	3	4	5		
Semitendinosus							
WBSF, kg	4.80 ^a	4.69 ^a	4.28 ^b	3.98 ^c	4.26 ^b	0.09	< 0.01
Proteolytic activity, a.u.							
Intact troponin-T	1.19	0.87	1.20	1.00	1.14	0.19	0.88
Degraded troponin-T	0.74	0.69	0.85	0.75	0.84	0.10	0.55
Longissimus lumborum							
WBSF, kg	3.16	3.35	3.41	-	-	0.08	0.88
Proteolytic activity, a.u.							
Intact troponin-T	0.49 ^b	0.86 ^{a,b}	1.01 ^a	-	-	0.15	< 0.01
Degraded troponin-T	0.87	0.72	0.92	-	-	0.11	0.68

^{a,b,c}Means lacking a common superscript within a row are different ($P < 0.05$).

¹ The ST was fabricated into 10 – 2.54 cm steaks, steak 1 being most proximal and steak 10 being most distal to the steers' body. Semitendinosus steaks were paired (1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10) and pairs were randomly assigned to 7, 14, 28, 56, and 112 d of aging. Steaks 1 and 2 comprised LOC 1, steaks 3 and 4 comprised LOC 2, steaks 5 and 6 comprised LOC 3, steaks 7 and 8 comprised LOC 4, and steaks 9 and 10 comprised LOC 5.

² Longissimus lumborum steaks were fabricated into 6 – 2.54 cm steaks and paired (1 and 2, 3 and 4, 5 and 6). Pairs were randomly assigned to 7, 28, and 112 d of aging. Steaks 1 and 2 comprised LOC 1, steaks 3 and 4 comprised LOC 2, and steaks 5 and 6 comprised LOC 3.

Table 3. Effect of four dietary treatments (**TRT**) on steak Warner-Bratzler shear force (**WBSF**), fiber type percentage and cross-sectional areas of Type I, Type IIA, and Type IIX muscle fibers, and intact and degraded troponin-T values in the Semitendinosus (**ST**) and Longissimus lumborum (**LL**).

	TRT				SEM ⁵	P-value
	CON ¹	ALG ²	AOX ³	LATE ⁴		
Semitendinosus						
WBSF, kg	4.41	4.34	4.30	4.55	0.12	0.41
Fiber type, %						
Type I	22.6	24.6	21.5	22.0	1.2	0.33
Type IIA	32.4	36.0	33.8	35.4	1.8	0.47
Type IIX	44.9	39.2	44.5	42.4	2.1	0.22
Cross-sectional area, μm^2						
Type I	2,314	2,532	2,408	2,582	123	0.43
Type IIA	2,859	2,944	2,819	2,869	173	0.96
Type IIX	3,621	3,924	3,629	3,555	187	0.52
Proteolytic activity, a.u.						
Intact troponin-T	1.30	1.08	0.90	1.04	0.19	0.01
Degraded troponin-T	0.59	0.82	0.89	0.80	0.10	0.15
Longissimus lumborum						
WBSF, kg	3.63 ^a	3.14 ^b	3.24 ^b	3.22 ^b	0.11	< 0.01
Fiber type, %						
Type I	29.5	32.3	29.3	28.3	1.1	0.08
Type IIA	31.7	34.1	34.4	31.0	1.5	0.28
Type IIX	38.6 ^{a,b}	33.4 ^b	36.1 ^{a,b}	40.5 ^a	1.7	0.03
Cross-sectional area, μm^2						
Type I	2,473	2,633	2,409	2,454	92	0.34
Type IIA	3,111	3,578	3,257	3,203	127	0.06
Type IIX	4,238 ^b	4,951 ^a	4,591 ^a	4,316 ^b	157	<0.01
Proteolytic activity, a.u.						
Intact troponin-T	0.84	0.67	0.47	1.18	0.21	0.40
Degraded troponin-T	0.75	0.88	0.81	0.92	0.13	0.98

^{a,b,c} Means lacking a common superscript within a row are different ($P < 0.05$).

¹ Consisted of steam-corn, wet-corn gluten feed, corn silage, ground flax, monensin and tylosin premix, mineral/vitamin supplement, ground limestone, salt, vitamin A, and vitamin E.

² Diet consisted of control diet fed with microalga supplement.

³ Diet consisted of control diet fed with microalgae meal supplement and added antioxidant mixture.

⁴ Diet consisted of control diet with mineral/vitamin supplement which did not contain selenium selenite and microalgae meal supplement fed in last 10 d of finishing.

Table 4. Sarcomere length across three aging periods¹ (DOA) in the Longissimus lumbarum (LL).

	Day of aging			SEM	DOA ³
	7	28	112		
Sarcomere length, μm	1.67 ^a	1.63 ^b	1.65 ^a	0.01	< 0.01

^{a, b, c} Means lacking a common superscript within a row are different ($P < 0.05$).

¹ Longissimus lumbarum steaks were aged for 7, 28, or 112 d postmortem at 2°C.

² Longissimus lumbarum steaks were fabricated into 6 – 2.54 cm steaks and paired (1 and 2, 3 and 4, 5 and 6). Pairs were randomly assigned to 7, 28, and 112 d of aging. Steaks 1 and 2 comprised LOC 1, steaks 3 and 4 comprised LOC 2, steaks 5 and 6 comprised LOC 3.

³ Main effect of DOA on sarcomere length.

Table 5. *P*-values of main effects and interactions for postmortem aging (**DOA**)¹, steak location (**LOC**), and dietary treatment (**TRT**) of Warner-Bratzler shear force (**WBSF**), sarcomere length, muscle fiber type percentages, cross-sectional area (**CSA**), desmin, troponin-T, and calpain activity in the Semitendinosus (**ST**).

	Linear DOA ¹	Quadratic DOA ¹	LOC ²	TRT ³	DOA ¹ × LOC ²	DOA ¹ × TRT ³	LOC ² × TRT ³	DOA ¹ × LOC ² × TRT ³
WBSF, kg	< 0.01	< 0.01	< 0.01	0.38	0.32	0.12	0.66	0.71
Sarcomere length, μm	0.02	0.02	< 0.01	0.56	< 0.01	0.40	0.39	0.28
Type I %	-	-	< 0.01	0.33	-	-	0.28	-
Type IIA %	-	-	< 0.01	0.47	-	-	0.58	-
Type IIX %	-	-	< 0.01	0.22	-	-	0.61	-
Type I CSA, μm^2	-	-	< 0.01	0.41	-	-	0.73	-
Type IIA CSA, μm^2	-	-	< 0.01	0.97	-	-	0.99	-
Type IIX CSA, μm^2	-	-	< 0.01	0.52	-	-	0.70	-
Intact desmin, a.u.	< 0.01	0.02	0.13	0.17	0.44	0.55	0.03	0.28
Degraded desmin-1, a.u.	< 0.01	< 0.01	0.67	0.89	0.23	0.99	0.88	0.95
Degraded desmin-2, a.u.	0.04	0.70	0.97	0.66	0.80	0.25	1.00	0.99
Intact troponin-T, a.u.	< 0.01	< 0.01	0.34	0.01	0.55	0.06	0.10	0.51
Degraded troponin-T, a.u.	< 0.01	0.05	0.88	0.15	0.62	0.23	0.41	0.59
Intact calpain-1, a.u.	< 0.01	< 0.01	0.55	0.75	0.88	0.95	0.01	0.35
Autolyzed calpain-1, a.u.	0.77	0.67	0.23	0.48	0.45	0.58	0.76	0.95
Intact calpain-2, a.u.	0.90	0.95	0.43	0.51	0.43	0.97	0.68	0.91
Autolyzed calpain-2, a.u.	0.41	0.41	0.74	0.79	0.59	0.83	1.00	0.95

¹Steaks from ST were aged 0, 7, 14, 28, 56, or 112 d postmortem at 2°C.

² The ST was fabricated into 10 – 2.54 cm steaks, steak 1 being most proximal and steak 10 being most distal to the steers' body. Semitendinosus steaks were paired (1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10) and pairs were randomly assigned to 7, 14, 28, 56, and 112 d of aging. Steaks 1 and 2 comprised LOC 1, steaks 3 and 4 comprised LOC 2, steaks 5 and 6 comprised LOC 3, steaks 7 and 8 comprised LOC 4, and steaks 9 and 10 comprised LOC 5.

³Steers were fed 4 dietary treatments a control (**CON**), control diet with microalgae meal supplement (**ALG**), control diet with microalgae meal supplement and antioxidant (**AOX**), and control algae diet until last 10 d of finishing, in which the diet was supplemented with the microalgae meal (**LATE**).

Table 6. *P*-values main effects and interactions for postmortem aging (**DOA**)¹, steak location (**LOC**), and dietary treatment (**TRT**) of Warner-Bratzler shear force (**WBSF**), sarcomere length, muscle fiber type percentages, cross-sectional area (**CSA**), desmin, troponin-T, and calpain activity in the Longissimus lumborum (**LL**).

	Linear DOA ¹	Quadratic DOA ¹	LOC ²	TRT ³	DOA ¹ × LOC ²	DOA ¹ × TRT ³	LOC ² × TRT ³	DOA ¹ × LOC ² × TRT ³
WBSF, kg	< 0.01	< 0.01	0.88	< 0.01	0.76	0.41	0.19	0.38
Sarcomere length, μm	< 0.01	< 0.01	0.83	0.99	0.91	0.82	0.61	0.76
Type I %	-	-	0.08	0.10	-	-	0.58	-
Type IIA %	-	-	0.28	0.29	-	-	0.10	-
Type IIX %	-	-	0.24	0.03	-	-	0.06	-
Type I CSA, μm^2	-	-	0.23	0.34	-	-	0.99	-
Type IIA CSA, μm^2	-	-	0.40	0.06	-	-	0.45	-
Type IIX CSA, μm^2	-	-	0.52	<0.01	-	-	0.70	-
Intact desmin, a.u.	0.03	0.15	0.96	0.19	0.96	0.77	0.60	0.73
Degraded desmin-1, a.u.	0.01	< 0.01	0.81	0.97	0.81	0.87	0.40	0.84
Degraded desmin-2, a.u.	0.04	0.07	0.38	0.79	0.15	0.57	0.99	0.98
Intact troponin-T, a.u.	0.02	0.01	< 0.01	0.40	< 0.01	0.57	0.24	0.06
Degraded troponin-T, a.u.	< 0.01	< 0.01	0.68	0.98	0.45	0.79	0.56	0.33
Intact calpain-1, a.u.	< 0.01	< 0.01	0.05	0.02	0.20	0.16	0.01	0.25
Autolyzed calpain-1, a.u.	0.47	0.48	0.91	0.86	0.53	0.44	0.98	0.81
Intact calpain-2, a.u.	0.84	0.88	0.78	0.74	0.66	0.34	0.90	0.60
Autolyzed calpain-2, a.u.	0.80	0.92	0.99	0.99	0.92	0.92	1.00	1.00

¹Steaks from LL were aged 7, 28, or 112 d postmortem at 2°C.

²Steaks from ST were divided into 5 steak locations with 2 steaks per location. Steak LOC 1 was the most proximal with steak LOC 5 as the most distal. Longissimus lumborum steaks were divided into 3 steak LOC, with 2 steaks per location. Steak LOC 1 was the most anterior and LOC 3 was the most anterior to the carcass.

³Steers were fed 4 dietary treatments a control (**CON**), control diet with microalgae meal supplement (**ALG**), control diet with microalgae meal supplement and antioxidant (**AOX**), and control algae diet until last 10 d of finishing, in which the diet was supplemented with the microalgae meal (**LATE**).

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Appendix A - Supplementary Data

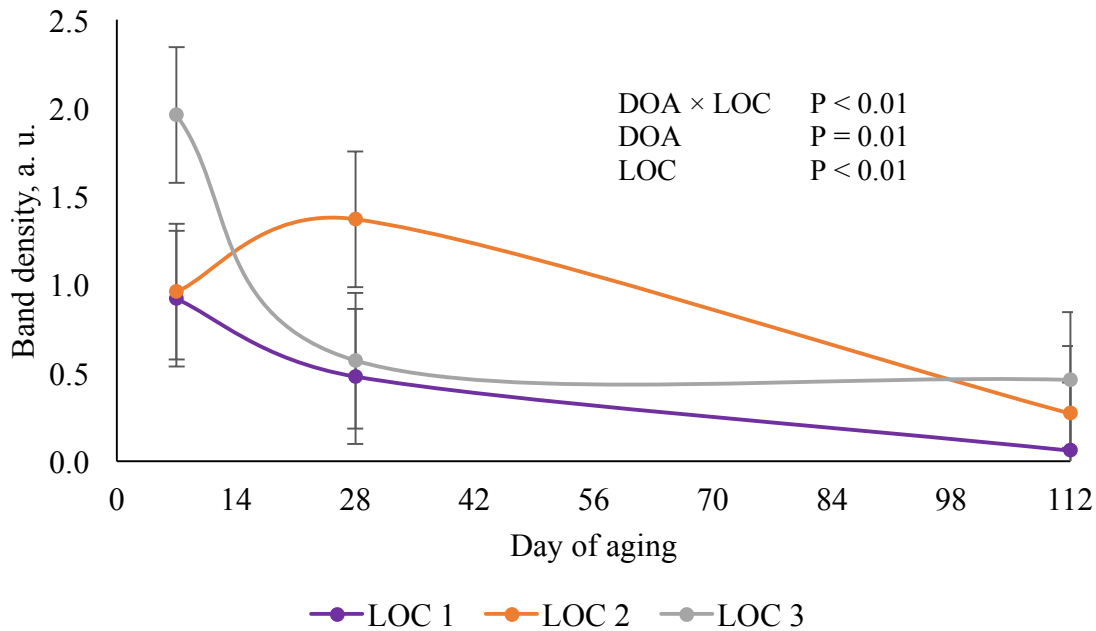


Figure 14. The interaction of postmortem day of aging (**DOA**) and steak location (**LOC**) of intact troponin-T in the Longissimus lumborum (**LL**). Immunoreactive bands located at 40 kDa were identified as intact troponin-T. Bands were equalized to a pooled sample on each blot. Longissimus lumborum steaks were fabricated into 6 – 2.54 cm steaks and paired (1 and 2, 3 and 4, 5 and 6). Pairs were randomly assigned to 7, 28, and 112 d of aging. Steaks 1 and 2 comprised LOC 1, steaks 3 and 4 comprised LOC 2, and steaks 5 and 6 comprised LOC 3. Longissimus lumborum steaks were aged at 2°C.

Table 7. Warner-Bratzler shear force values for five locations (LOC)^{1,2} in the Semitendinosus (ST) and three locations in the Longissimus lumborum (LL) across four dietary treatments (TRT)³.

Attribute	Treatments					TRT	LOC	TRT × LOC
	CON ¹	LATE ²	ALG ³	AOX ⁴	SEM			
WBSF, kg								
ST location								
1	4.69	5.10	4.74	4.62	0.17	0.38	< 0.01	0.66
2	4.83	4.86	4.54	4.50				
3	4.19	4.47	4.15	4.28				
4	4.08	3.94	3.93	3.93				
5	4.26	4.39	4.20	4.17				
LL location								
1	3.16	3.09	2.99	3.03	0.15	< 0.01	0.88	0.19
2	3.35	3.26	3.05	3.49				
3	3.41	3.32	3.38	3.22				

^{a,b,c}Means lacking a common superscript within a row are different ($P < 0.05$).

¹The ST was fabricated into 10 – 2.54 cm steaks, steak 1 being most proximal and steak 10 being most distal to the steers' body. Semitendinosus steaks were paired (1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10) and pairs were randomly assigned to 7, 14, 28, 56, and 112 d of aging. Steaks 1 and 2 comprised LOC 1, steaks 3 and 4 comprised LOC 2, steaks 5 and 6 comprised LOC 3, steaks 7 and 8 comprised LOC 4, and steaks 9 and 10 comprised LOC 5.

²Longissimus lumborum steaks were fabricated into 6 – 2.54 cm steaks and paired (1 and 2, 3 and 4, 5 and 6). Pairs were randomly assigned to 7, 28, and 112 d of aging. *Means are different ($P < 0.05$) within dietary treatment. Steaks 1 and 2 comprised LOC 1, steaks 3 and 4 comprised LOC 2, and steaks 5 and 6 comprised LOC 3.

Consisted of steam-corn, wet-corn gluten feed, corn silage, ground flax, monensin and tylosin premix, mineral/vitamin supplement, ground limestone, salt, vitamin A, and vitamin E.

² Diet consisted of control diet with mineral/vitamin supplement which did not contain selenium selenite and microalgae meal supplement fed in last 10 d of finishing.

³ Diet consisted of control diet fed with microalgae supplement.

⁴ Diet consisted of control diet fed with microalgae meal supplement and added antioxidant supplement.

Table 8. Interaction of dietary treatment (TRT) and steak location (LOC)¹ on intact, degraded desmin – 38 kDa, and degraded desmin – 35 kDa of Semitendinosus (ST) steaks fed a control (CON)² diet, control diet plus microalgae supplement (ALG)³, control diet plus microalgae supplement and antioxidant mixture (AOX)⁴, and control diet plus microalgae supplement fed within the last 10 d of feeding (LATE)⁵. Treatments were examined across five LOC¹ within the ST.

Attribute	Treatment				LOC ^{1,7}	SEM	P-value		
	CON ²	ALG ³	AOX ⁴	LATE ⁵			TRT	LOC ¹	LOC ¹ × TRT
Intact desmin, a. u.									
Semitendinosus location ⁵									
1	1.35	2.10	0.97	1.44	1.46	0.43	0.17	0.13	0.03
2	0.88	0.83	0.83	0.99	0.88				
3	1.08	2.53	0.58	0.87	1.26				
4	2.23	0.61	1.46	0.83	1.28				
5	1.09	1.59	0.60	0.82	1.03				
Treatment ⁶	1.33	1.53	0.89	0.99					
Degraded desmin – 38 kDa, a.u.									
Semitendinosus location ⁵									
1	2.24	2.30	1.79	1.42	1.94	0.75	0.89	0.67	0.88
2	1.08	0.96	1.15	1.20	1.10				
3	1.59	1.05	0.80	2.52	1.49				
4	2.50	1.84	1.83	2.86	2.26				
5	1.76	1.59	1.54	1.43	1.58				
Treatment ⁶	1.83	1.55	1.42	1.89					
Degraded desmin – 35 kDa, a.u.									
Semitendinosus location ⁵									
1	0.69	1.15	1.75	0.44	1.01	0.63	0.66	0.97	1.00
2	0.28	0.98	1.46	0.38	0.77				
3	0.94	0.72	1.85	1.15	1.17				
4	0.87	1.10	0.88	0.45	0.82				
5	1.63	2.28	1.11	1.21	1.56				
Treatment ⁶	0.88	1.25	1.41	0.73					

¹The ST was fabricated into 10 – 2.54 cm steaks, steak 1 being most proximal and steak 10 being most distal to the steers' body. Semitendinosus steaks were paired (1 and 2, 3 and 4, 5 and 6, 7 and 8, 9 and 10) and pairs were randomly assigned to 7, 14, 28, 56, and 112 d of aging. Steaks 1 and 2 comprised LOC 1, steaks 3 and 4 comprised LOC 2, steaks 5 and 6 comprised LOC 3, steaks 7 and 8 comprised LOC 4, and steaks 9 and 10 comprised LOC 5.

²Consisted of steam-corn, wet-corn gluten feed, corn silage, ground flax, monensin and tylosin premix, mineral/vitamin supplement, ground limestone, salt, vitamin A, and vitamin E.

³Diet consisted of control diet fed with microalgae supplement.

⁴Diet consisted of control diet fed with microalgae meal supplement and added antioxidant mixture.

⁵Diet consisted of control diet with mineral/vitamin supplement which did not contain selenium selenite and microalgae meal supplement fed in last 10 d of finishing.

⁶Mean of main effect TRT across 5 steak locations.

⁸Mean of main effect LOC across 4 diet treatments.

